OBSERVATIONS ON THE HAEMOCYTES OF CERTAIN INSECT PESTS AFFECTED BY SOME CHEMICALS

THESIS

SUBMITTED FOR THE AWARD OF THE DEGREE OF

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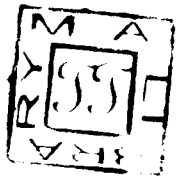

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Certified that the work entitled ***"Observations on the haemocytes of certain insect pests affected by some chemicals"*** *has been carried out under the supervision of Prof. Mumtaz Ahmad Khan and subsequent to his retirement completed under supervision of the Chairman, Department of Zoology, AMU. The work is original and has been independently persued by the candidate.*

I permit the candidate to submit the work for the award of the degree of Doctor of Philosophy in Zoology of Aligarh Muslim University, Aligarh, India.

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(AYESHA QAMAR)

I- INTRODUCTION

The blood or haemolymph of insects is a nearly neutral fluid consisting of a serum fraction in which there are free floating cells called haemocytes. Insects have been known to possess white blood cells (haemocytes), since the time of Swammerdam (1637-1680). Their versatile features in a species and reduplication of similar and compatible shapes amongst different species urged Cue'not (1896) to classify them for the first time into four different categories (Millara 1947). Tauber and Yeager (1934) and Jones (1964) suggested that haemocytes of insects are comparable in their morphology, embryonic origin, amoeboid movements and phagocytic activity to white blood cells of mammals. Wigglesworth (1959) reviewed functions of these cells as phagocytosis and immunity, protection from metazoan parasites, coagulation, intermediary metabolism and connective tissue formation. Yeager and coworkers (1942) suggested one more function, that of detoxication which was later confirmed by Patton (1961).

Insect haemocytology has developed slowly and sporadically over the years. Substantial advancement in the established knowledge of insect haemocytes is the result of the steadily increasing accumulation of recent research, due to the increased interest of workers involved in the subject and the development of new techniques (Khalifa and Siddiqui 1985). A knowledge of normal haemocytes of an insect is necessary to physiologists, toxicologists and biochemists, as alterations in structure, types and number of cells reflect changes in physiological and biochemical processes. Abnormal changes may be due to poisons and diseases.

During the lifetime, insect may face exposure to toxins and poisons of their predators as well as to all sorts of chemical poisons . Relatively little information is available on the specific response of insect haemocytes or haemolymph to poisons. Several reviews, carried out recently, on the insect haemocytes unfortunately lack information on the behavior of various blood cells in ordinary circumstances or under stressed conditions. Although some experiments seem to indicate detoxifying role for the haemocytes most of the literature states or implies that the observed haemocyte responses are principally attributable to death or degeneration of the

cells. There seems to be little, if any, clear cut evidence for defence reaction of haemocytes against toxins. The changes in response to insecticides as observed by some authors, after blockage of haemocytes, could result from the deleterious effects of the breakdown products of the haemocytes as well as from the loss of a detoxifying role of haemocytes. Virtually nothing is known about the direct role of haemocytes in the detoxification of poisons. However, an indirect role of haemocytes in detoxification mechanism via esterases is possible (Patton 1961). Patton suggested that non specific esterases in haemocytes might be involved in detoxifying parathion. Witten (1968) also suggested an active role of haemocytes in detoxication of contact insecticides that penetrate the tarsal cuticle in the fly *Sarcophaga bullata*. Yeager and coworkers (1942) have also reported that haemocytes detoxify poisons.

The contribution of haemocytes in prophenoloxidase system through the involvement of granulocytes and/or plasmatocytes prompted Gupta (1986) to label these two categories of haemocytes as immunocytes. He suggested that arthropod granulocytes and plasmatocytes are functionally comparable to mammalian T and B lymphocytes and granulocytes are functional hybrid of both T and B lymphocytes. However types of immunocytes and their role in insects are debatable and need more information on the subject for clarity (Hazarika and Gupta 1987, Han and Gupta 1989)

The present study is aimed at the documentation of various events regarding the cell morphology in general, total haemocyte counts and differential haemocyte counts following the topical treatment of various lethal as well as sub-lethal concentrations of various insect control agents to the red cotton bug *Dysdercus cingulatus* and the Bihar hairy caterpillar *Diacrisia obliqua*. These two test insects belong to two different groups i.e. hemimetabola and holometabola, respectively, having incomplete and complete metamorphosis. Changes, if any, in the haemocyte structure were also examined at ultrastructural level under the Transmission Electron Microscope (TEM). The insecticides included in this study were selected from different chemical categories viz., organophosphate (Acephate) carbamate (Aminocarb) pyrethroid (Cypermethrin) ,juvenoid (Methoprene) and ecdysteroid

(Muristerone). The objectives of the present investigation were four fold (i) whether the chemicals belonging to different categories would bring about specific kind of cytological abnormalities in the haemocytes of *D. obliqua* and *D. cingulatus* (ii) whether these chemicals bring about the changes in the population of circulating haemocytes. (iii) whether different concentrations of these chemicals would bring about different pathological effects, if any, in different insects under similar conditions (iv) and finally what are the short term effects of treatment of these chemicals on haemocytes i.e. changes induced in haemocyte population and morphology within the same stadium on which treatment was made; and long term residual effects i.e. Changes in haemocytes after the insects moulted to next instar.

Since haemocytes play very important part primarily in insect immunity which is accomplished by phagocytosis, encapsulation, nodule formation, secretion of immunologic factors, coagulation and poison detoxification mechanisms (Gupta 1986) and secondarily in the intermediary metabolism of fat, proteins and carbohydrates, thus any destructive effect on haemocytes might render these tasks unfulfilled, consequently interfering with the normal physiological processes.

The present observations may prove significant for understanding the efficacy of chemicals belonging to different categories of insecticides and insect growth regulators on different types of haemocytes of insects and their manifestation on the overall physiology of insects. This study is also important in connection with the analysis of blood cells following insecticide application for the insect control. The present work will also prove vital in future studies regarding the analysis of the behaviour of the haemocytes in response to different chemicals and other control agents used in Integrated Pest Management (IPM).

II- REVIEW OF LITERATURE

The freely circulating haemocytes of a variety of insect species belonging to different orders have been extensively studied by many workers. They have identified and classified various types of haemocytes on the basis of staining reactions, morphology, functions and histochemical properties. A number of authors have already given detailed reviews concerning classification of insect haemocytes as studied under the light microscope (Poisson and Pesson 1939; Yeager 1945; Wigglesworth 1959 and 1973; Jones 1962, 1964 and 1977; Gupta 1979; Arnold 1974, 1982 and 1985; Ahmad 1986; Siddiqui 1990; Cebesoy and Ayvali 1996). According to available information substantial disagreement exists not only regarding the numbers but the classification of haemocytes also, because of inconsistencies in nomenclature, experimental techniques or in the use of insects of different developmental stages. Thus uniform identification of various haemocyte types is difficult. This situation is further complicated by the diversity and variation in the morphological features of the cells within a single insect at any given time (Joshi and Lambdin 1996). In the last couple of decades studies by electron microscope have proved helpful in distinguishing various haemocyte types (Lai-Fook 1970, 1973; Akai and Sato 1971; Neuwirth, 1973; Rowley and Ratcliffe 1981; Kim 1981; Gupta 1979, 1985 a, 1986 and 1991; Beeman, *et al* 1983; Essawy *et al* 1985; Butt and Shields 1996; Joshi and Lambdin 1996).

An extensive account of the haemocytes of insects dealing with differentiation morphology, histochemistry, techniques, variation in numbers, functions, ultrastructure and effect of chemicals has been published by respective specialists in a book entitled "Insect haemocytes" edited by A. P. Gupta (1979). A detailed study on haemocytic immunity, humoral immunity and techniques as well as biomedical applications has also been published by the same author in 1986. In addition to these, several recent reviews are also available regarding biochemical aspects of immunity (Dunn 1986); haemocyte behaviour (Lackie 1986); immune reactions in *Drosophila* and other insects (Hultmark 1993); cellular defense responses of insects (Ratcliffe 1993); innate immunity of insects (Hoffmann 1995) and biological mediators of insect immunity (Gillespie *et.al.*1997).

Research on the effects of chemicals on haemocytes started in early 20th century when Mc Indoo (1917) observed that prolonged exposure to nicotine vapours induced vacuolization in haemocytes of *Apis mellifera*. Tareeva and Nenyukov (1931) reported that in grass hopper, *Calliptamus italicus* abnormally large haemocytes appeared after sodium arsenate poisoning. They also recorded the disintegration and destruction of the haemocytes. In 1932, Shull *et al* studied the effect of 34 toxic gases on the blood of cockroach, *Periplaneta americana*. These gases were ammonia, hydrochloric acid, carbon disulphide, methyl alcohol, carbon tetrachloride, chloroform, 1,2-dichloroethane, trichloroethylene tetrachloroethylene, diethyl ether, acetic acid, amylacetate, hydrogen cyanide, benzene, nitrobenzene, chlorobenzene, paradichlorobenzene, cyclohexane, bromocyclohexane, methyl cyclohexane, xylene (U.SP), cymene, toludine, dimethylaniline, diethyl aniline, methyl salicylate, pyridine, nicotine, naphthalene, tetrahydronaphthalene, linonine and d- camphor. It was found that cockroaches exposed to carbon disulphide yielded little blood which contained only a few cells in proportion to the volume of blood. These cells were much more granular than those found in untreated insects and in insects which were exposed to other compounds, but no morphological changes were observed. Insects treated with pyridine also yielded little blood but the cell content was apparently normal. Pilat (1935) studied the effect of intestinal poisoning with sodium arsenite and sodium silicofluoride on the blood of *Locusta*. Both Shull *et al* (1932) and Pilat (1935) inferred that the influence of the poison on blood of insects was far from discoverable in all cases. Even in the presence of unmistakable signs of poisoning and when the poisoned insects died, the picture of blood in most cases did not present any appreciable deviation from its normal condition, however, in some cases disintegration and destruction of haemocytes took place. Moreover, mitotic figures of nuclei were frequently found. Fisher (1936) observed the effects of a few toxic substances (arsenic containing 99% arsenous acid anhydride (As_2O_3), mercuric chloride, sodium fluosilicate, hydrocyanic acid, ether, pyridine and carbon disulphide upon the total blood count in the cockroach *Blatta orientatis*. Arsenic, mercuric chloride and sodium fluosilicate caused respectively significant decrease from the acetic acid (Fisher, 1935) average of 36,173 to 8,179, 7083 and 6,778 cells per cubic mm of blood. The reduction was apparently caused by the disappearance of the type of cells having a small nucleus, and a large amount of cytoplasm which were normally present in large

numbers. Carbon di-sulphide had no apparent effect upon the blood cell count. Hydrocyanic acid and ether showed no difference from acetic acid in their effect upon the blood cell count. Lepesme (1937) came to conclusion that arsenicals caused changes in the haemocytes. When sodium arsenite was contact applied to *Schistocerca gregaria*, the cells responded by mitosis, vacuolization, chromatolysis and eventually complete breakdown. On the other hand, Woke (1940) reported no histo-pathological changes in the haemocytes of the larvae of *Prodenia eridania* which were orally poisoned by phenothiazine.

Yeager and Munson (1942) made a detailed study on the changes induced in the blood cells of the Southern armyworm *Prodenia eridania* by the administration of poisons viz. nicotine bentonite, nicotine peat, rotenone, pyrethrum, phenothiazine, barium fluosilicate, sodium fluoaluminate, sodium fluoride, mercuric chloride, calcium arsenate, calcium arsenite, arsenic trioxide, Paris green and lead arsenate. These authors concluded that marked haematological changes followed the administration of nicotine bentonite, nicotine peat, rotenone, pyrethrum and phenothiazine. Furthermore, marked haematological changes in the fore ends relative to hind ends of the ligatured larvae followed the administration of the arsenicals, fluorides and mercuric chlorides. Moreover, an increase of mitosis seemed to occur soon after administration of the arsenicals, fluorides and mercuric chloride. The haematological changes that followed poison administration occurred progressively. The degenerative cytoplasmic changes consisted of apparent cellular swelling, disruption of and decrease in visibility of normal structure, achromophilia, decrease or loss of blood cell glycogen, formation of broad pseudopodia or cytoplasmic bulgings, plastid formation, excessive vacuolization and raggedness. Whereas, nuclear degeneration involved distortion, raggedness, loss or disruption of normal structure, achromophilia, assumption of more or less peripheral position fragmentation, pycnosis and extrusion. In *Periplaneta americana* (Yeager *et al.*, 1942), Chinese ink loaded haemocytes did not perform normal functions and increased mortality of roaches after application of metasodium arsenite and nicotine. They further interpreted that haemocytes normally detoxify insecticides. The haemocytes of *Leptinotarsa decemliniata* developed nuclear changes and cytolysis following oral dose of DDT, whereas, injection of BHC caused abnormal vacuolization and cytolysis (Arvy *et al.*, 1950).

A comparative study was also performed on the effect of three fumigants viz. dichloroethyl ether, carbon tetrachloride and methyl bromide on the haemocytes of the larvae of Mediterranean flour moth *Ephestia kuhniella* by Arnold (1952b). The cytological changes in the haemocytes, their total number, and the relative number of cells in each class were noted at intervals during fumigation and in recovering and moribund larvae after treatment with each compound. Fumigation caused passive active cell changes and pathological conditions leading to cell degeneration and also resulted in significant decrease in cell numbers. Total haemocyte number decreased further in individuals that failed to recover from fumigation. However, recovery was associated with regenerative changes that resulted in increased cell number, chiefly by spheroidocytes which were loaded with the globules of neutral fat at that time. Arnold suggested that spheroidocytes were associated with recovery through an ability to aid in the creation of fat reserves for energy metabolism under favourable conditions.

However, Hopp (1953) concluded that application of carbon tetrachloride and elthylene trichloride on the body louse *Pediculus humanus* resulted in nuclear enlargement and swelling of chromatin in the haemocytes, but the prolonged effect of poisoning caused lysis of both nuclei and cytoplasm. Jones (1957) summarized that phagocytic haemocytes (Plasmatocytes) slightly but significantly decreased during DDT poisoning in unfixed treated meal worm larvae and remained so prior to the moribund stage. Heat fixation of these larvae significantly altered the blood picture by obscuring the distinguishing features between the two main kinds of cells (plasmatocytes and cystocytes). Total haemocytes counts in the unfixed and the heat fixed larvae following DDT treatment were within the normal range prior to the moribund stage. It was concluded that haemocytes played no role in natural defenses of mealworm against DDT.

Chattoraj and Sharma (1964) working on about a dozen insecticides and four insects found similar types of pathological changes in the haemocytes of grass hopper and cockroach. Furthermore, changes found in the haemocytes of *Spodoptera* larvae were common with those of *Atteva* i.e. no specific insecticidal action was noted. The pathological changes of the haemocytes were of general type. The affected haemocytes of grasshopper and cockroach showed formation of microhaemocytes,

megalohaemocytes, abnormal granulation, vacuolation, cytoplasmic and nuclear extrusion, whereas, in case of affected lepidopterous larvae there was shrinkage in cytoplasm with formation of pseudopodia like structure and fusiform cells turned roundish. The insecticides viz, aldrin, dieldrin, dimecron, lindane, methoxychlor and nuvan had little effect on the haemocytes of these species. On the other hand, parathion, endrin, sevin and allethrin caused intense pathological damage to the haemocytes of all affected insect.

Gupta and Sutherland (1968) studied the effect of sublethal doses of chlordane on the haemocytes and midgut epithelium of *Periplaneta americana*. They found that chlordane caused some apparent increase in total haemocyte count in treated cockroaches. This increase although not statistically significant indicated that chlordane in sublethal amounts stimulated greater release of haemocytes into the haemolymph and thereby correspondingly plasmatocytes, granular haemocytes, spherule cells and cystocytes increased in number. They further stated that although statistically insignificant, there was greater cellular degeneration in plasmatocytes, granulocytes and spherule cells.

The nuclear vacuolation was mentioned by Bandhopadhyay (1970) in the haemocytes of cockroach *Periplaneta americana* by the application of malathion. Roy and Bagchi (1973) aimed to find out changes brought about in cellular structure of the haemocytes of *P. americana* by the application of parathion, endrin, and thiodan. The changes in the structure of haemocytes after treatment were almost the same with respect to all the three insecticide. The general nature of changes in the haemocytes were cytoplasmic vacuolation, accumulation of coarse stained granules, formation of pseudopodia, breakage of cell wall and extrusion of granules. Whereas, nuclear injury consisted of dissolution of nuclear membrane, dislocation, pycnosis and in extreme case of damage, there was complete breakage of nucleus leaving a lump of chromatin. In a very few cases the initiation of mitosis was noted. In some smears the degeneration gradually increased with the increase in concentration and time after exposure. Plasmatocytes were found to be mostly affected by the action of all the insecticides. Parathion produced the most conspicuous and intense haematological changes, an observation, which was in agreement with the work of previous authors.

However, they concluded that parathion did not cause agglutination of haemocytes, as described by Chattoraj and Sharma (1964). Bhargawa and Pillai (1976) observed the haematological effect of apholate which significantly reduced the number of plasmatocytes in the adults of *Dysdercus koenigii*.

Zaidi and Khan (1977) investigated the effect of topical application of technical aldrin (chlorinated hydrocarbon) and dipterex (organophosphate) on the haemocytes of the red cotton bug *Dysdercus cingulatus* and recorded that all types of haemocytes were pathologically affected. In general, dipterex was more harmful to haemocytes than aldrin. The adipohaemocytes and granular haemocytes were the most susceptible even to dilute concentration of dipterex. The oenocytoids were highly resistant and were only destroyed by the strongest concentration of dipterex (2%). Occasionally, some plasmatocytes indicated recovery from the pathological effect of dipterex. Abnormal haemocytes showed cytoplasmic extensions, abnormal vacuolization, ragged achromophilic and scattered cytoplasm, dislocation of nucleus, fragmentation and swollen nuclei.

Behura and Dash (1978) investigated the effect of ingestion of insecticides such as dimethoate, fenitrothion, parathion, methyl demeton, thiometon and phosphamidon each at 0.01% concentration on haemocytes of *Rhopalosiphum maidis*. These compounds, except phosphamidon appeared to have no visible effect on the haemocytes. But phosphamidon had direct effect on the haemocytes of the aphid species which was manifested by breakage of plasma membrane, fragmentation of nucleus, formation of vacuoles, shrinkage of cell membrane etc. No changes, however, could be discerned in the prohaemocytes and according to them it was probably because the nucleus is large and occupies almost the entire space of the cell. Farks (1984) observed that mitosis could be induced in the haemocytes by injecting cholestrol, hydrocortisone, (cortisol) or 10% solution of glucose in *Galleria mellonella* and *Tenebrio molitor* but not by ergocalciferol.

Ahmad and Khan (1987) observed the effect of DDT (chlorinated hydrocarbon) and furadan (carbamate) on the haemocytes of the larvae of *Spilosoma obliqua*. They reported that topical application of sublethal concentrations of technical DDT and

furadan on the 3rd instar larvae caused pathological conditions in all types of haemocytes of the successive larval instars as well as in those of prepupal and pupal stage. By each concentration of both the insecticides the order of intensity of damage was adipohaemocytes > podocytes > plasmatocytes > oenocytoids. The oenocytoids were the most resistant cells and were completely changed only by the prolonged effect of the strongest concentration of each insecticide. Comparatively, the sublethal concentrations of furadan were more toxic to these cells than those of DDT. The authors further explained that the pathological symptoms in the affected haemocytes were generally initiated with pushing of the nuclei to eccentric position, abnormal vacuolization and granulation in the cytoplasm, breaking of nuclear membrane, discharge of swollen nuclei, fragmentation of chromatin material, cytoplasmic bulging and irregular as well as folded cell membrane. Finally, the entire cytoplasmic contents of the cell streamed out.

Green and Carter (1991) reported that exposure of 3rd instar larvae of *Tipula paludosa* to ethyl ether resulted in a 1.6 fold increase in total haemocyte count but exposure to acetic acid did not produce a significant increase in haemocyte number. Exposure to both vapours resulted in a 2.8 fold increase in total haemocyte count, with the majority of additional haemocytes being amoeboid and spindle shaped plasmatocytes which increased by 3.5 and 19.3 fold, respectively. According to them dehydration did not appear to be a major cause of increased haemocyte population.

Beside insecticides, the effect of hormones, plumbagins and antitumor drug was also observed on the haemocytes. Judy and Marks(1971) for the first time attempted to observe the effect of ecdysterone (a synthetic analogue of moulting hormone) on the haemocytes of *Manduca sexta, in vitro*, and reported that the migratory activity of spherule cells and plasmatocytes increased. According to Nishi, (1982) the haemocytes of the larvae as well as the pupae of *Spodoptera litura* were pathologically affected following the injection of different doses of β-ecdysone. Pathological symptoms were initiated with dislocation of the nucleus, cytoplasmic and nuclear vacuolization and irregularity in the shape of the cells. The pathological conditions increased progressively from lower to higher concentrations of β-ecdysone/larva.

Rao *et al.*, (1984) studied the effect of ligation and ecdysone on the total haemocyte counts in the tobacco caterpillar, *Spodoptera litura*. In the late 5th instar larvae, ligation just behind the thorax resulted initially in a decrease in the THC in both anterior and posterior halves, but 48 hrs post ligation, cell number increased in the anterior half. Such situation, according to them, was probably because of the presence of thoracic glands in the anterior region and also the elaboration of new cells from the anteriorly situated haemopoetic organs. They conformed the former possibility by injection of 50 μg of β-ecdysone into the hormone deficient posterior half resulting in a significant increase in the haemocyte count, presumably because of direct or indirect hormonal stimulation of mitosis in circulating haemocytes. Mitosis as a factor to increase the haemocyte number by ecdysterone was again confirmed by Farks (1984) when he injected different concentrations of the hormone into the larvae of penultimate and final instars of *Galleria mellonella* and *Tenebrio molitor* and found induced mitosis in plasmatocytes and prohaemocytes respectively and the highest mitotic index value was observed 6-12 hours after the injection of the hormone.

Contrary to the above observations of Rao *et al.*, (1984) and Farks (1984) when 5th instar hopper of *Hieroglyphus nigrorepletus* were injected with different sublethal doses of Triol (synthetic analogue of ecdysone) and Makisterone-A (phytoecdysone) by Ahmad and Khan (1988), both ecdysteroids destroyed the haemocytes. Triol was more effective to annihilate the haemocytes of these hoppers as compared to Makisterone A. They concluded that these hormones in sub-lethal doses, produced pathological symptoms in all types of haemocytes in the same stage and generation on which the hormone was applied as well as on the subsequent generation leading to depletion of total haemocyte counts as well as differential haemocyte counts.

Sujatha *et al.*, (1991) reported that starvation during larval development prevented the normally occurring increase in the total haemocytes number in *Corcyra cephalonica*. This effect was reversed either by resumption of feeding (on crushed sorghum seeds) or by the topical application of 20-hydroxyecdysone (ecdysterone). Pathak (1991) studied the effects of diuretic hormone, antiduretic hormone and juvenile hormone on *Halys dentata* adults. Diuretic hormone decreased the blood volume and consequently there was an increase in THC. However, no effect was

observed on DHC or absolute blood cell count. Antidiuretic hormone increased the blood volume thereby decreasing the cell count occurred. Similarly, no change in DHC or in absolute number of circulating haemocytes was recorded. Juvenile hormone had no effect on blood volume. However, the THC and absolute count increased, which was, according to them interpreted that juvenile hormone was capable of mobilizing non-circulating sessile haemocytes.

Siddiqui (1990) studied the effects of anti-tumor drug deoxyphylotoxin, currently known as insecticide on haemocytes of *Leptocorisa varicornis* and *Nepa cinerea* and found that effects were dose and period of exposure based. The most susceptible haemocytes were cystocytes followed by adipohaemocytes, prohaemocytes granulocytes, plasmatocytes and oenocytoids. The effect of plumbagin, isolated from *Plumbago zeylanica* on the haemocytes of adults of *Dysdercus koenigii* was determined using Scanning Electron Microscope (Tikku *et al.*, 1992). All 5 types of haemocytes were affected within a period of 24-48 h. The effect began with an acute state of vacuolization of the affected cells and a gradual destruction of cellular organelles, followed by dissolution of plasma membrane and evacuation of internal organelles (such as mitochondria and endoplasmic reticulum). They concluded that this phenomenon was responsible for a consistent elimination of the haemocytes from the blood leading to decrease in resistance of *D. koenigii* to plumbagin and subsequent mortality.

III- MATERIALS AND METHODS

(i) Breeding and maintenance of stock culture of *Dysdercus cingulatus*.

Adults and nymphs of *Dysdercus cingulatus* were collected from the cotton and lady finger fields during the months of March and April. These were maintained in glass rearing jars measuring approximately 20x15 cm containing a layer of 4 cm thick moist and coarse sand, which was previously sterilized at high temperature, at the bottom. The mouth of these jars was covered with a piece of muslin cloth fixed with a rubber band. These rearing jars were kept at 28 ± 2 °C and 70-80 % relative humidity (RH) in a BOD incubator. All the stages were fed on freshly soaked healthy cotton seeds. Over crowding was avoided.

(ii) Breeding and maintenance of stock culture of *Diacrisia obliqua*.

Adult moths of *Diacrisia obliqua* were collected from the lamp posts in the months of September and October. These adults were maintained in glass rearing jars measuring approximately 20 x 15 cm. The mouth of these jars was covered with a piece of muslin cloth which was tightly fixed by means of a rubber band. These jars were kept in a B.O.D. incubator cabinet maintained at 28 ±2 °C and 70-80 % R.H. Adult moths were fed on saturated glucose solution (Hashmat and Khan 1970). For this purpose, a piece of sterilized cotton wool was wrapped around a glass slide and was soaked with fresh glucose solution. Such slides were obliquely placed against the jar wall. The females laid eggs on folded paper strips 2" x 20" kept in the jars. Hatching took place after about a week. After hatching the larvae were fed on fresh castor (*Ricinus communis*) leaves. The fully matured larvae pupated at the surface of the jars or at the under surface of leaves. The emergence of adults took place after a

week following pupation. The newly emerged adults were subsequently transferred in pairs to separate rearing jars for further generations.

(iii) Sampling of experimental insects

The *Dysdercus cingulatus* bears five nymphal instars and after which adults emergence takes place, whereas, *Diacrisia obliqua* has 6 larval instars followed by a pupal instar which subsequently undergoes ecdysis and transforms into adult. In the present experiments, last nymphal instar (5^{th} instar) of *Dysdercus cingulatus* and last larval instar (6^{th} instar) of *Diacrisia obliqua* were used for observations. For this purpose, with respect to *Dysdercus cingulatus*, freshly moulted 4^{th} instar nymphs were isolated between 10:00 am and 12:00 noon each day and maintained age wise. Similarly, freshly moulted 5^{th} instar larvae of *D. obliqua* were also isolated between 10 am and 12 noon and maintained age wise. Subsequently, 5th instar nymphs of *D. cingulatus* and 6^{th} instar larvae of *D. obliqua* were treated with different concentrations of selected insecticides as described below. The moulting of nymphs and larvae were visually ascertained by the shed exuvae and size of the instar at the above mentioned time. At that time these nymphs or larvae were considered to be of zero age. After one day (24 hrs) following zero age i.e. w. e. f. 12:00 noon. nymphs or larvae were treated with the selected chemicals.

(iv) Dilution and application of chemicals

In the present investigation five compounds of different chemical nature namely Acephate (0, S-dimethyl acetyl phosphoramidothioate); Aminocarb (4-(dimethyl amino)-3-(methyl phenyl methyl carbamate), Cypermethrin (cyano(3-phenoxyphenyl) methyl 3-(2,2-dichloroethenyl)-2,2-dimethyl cycloprppanecarboxylate); Muristerone (5β.7-cholestene-2β.5a.14,20,22-heptol-6-one) and Methoprene ([Isopropyl (2E. 4E. 7S)-11-methoxy-3.7.11-trimethyl-2,4-dodecadienoate]) were used for topical application on 1-2 day old 5^{th} instar nymphs and 6th instar larvae of *D. cingulatus* and *D. obliqua* respectively. The effects of these chemicals were recorded on mortality.

moulting, haemocytes morphology, Total Haemocyte Counts (THC) and Differential Haemocyte Counts (DHC). For both the nymphal and larval stages of *Dysdercus cingulatus* and *Diacrisia obliqua*, the stock solutions of organophosphate, carbamate, synthetic pyrethroid, ecdysteroid and juvenoid were further diluted. The various concentrations of these chemicals for *Dysdercus cingulatus* were as follows:

Acephate:	0.006%, 0.004%, 0.002%, 0.001%.
Aminocarb:	0.008%, 0.007%, 0.004%, 0.0025%.
Cypermethrin:	0.001%, 0.0008%, 0.0006%, 0.0004%.
Muristerone:	0.8%, 0.6%, 0.4%, 0.2%.
Methoprene:	0.2%, 0.1%, 0.08%, 0.04.

Similarly, for *Diacrisia obliqua*, the stock solutions of organophosphate, carbamate, synthetic pyrethroid, ecdysteroid and juvenoid were further diluted as:

Acephate:	0.2%, 0.1%, 0.08%, 0.04%.
Aminocarb:	0.6%, 0.4%, 0.2%, 0.1%.
Cypermethrin:	0.015%, 0.01%, 0.005%, 0.0025%.
Muristerone:	2.0%, 1.5%, 1.0%, 0.5%.
Methoprene:	1.0%, 0.8%,0.6%, and 0.4%.

From each concentration of diluted insecticides, only 1 μl was applied topically on the meta-thoracic pleuron of individual 5th instar nymphs of *D. cingulatus*. Whereas 2 μl solution of each diluted concentration was applied on the individual 6th instar larvae of *D. obliqua* by 26 gauge needle attached to a tuberculin syringe fitted into a manually operated microapplicator. At the time of topical application of insecticides, the age of experimental insects was approximately one-two days. For each concentration 100 nymphs or larvae in four replicates, each consisting of 25 individuals, were used. Two controls consisting of 100 nymphs/larvae of same age were also run parallel to each series of experiments. One control group was treated with acetone (solvent) only while the other had untreated nymphs or larvae of the same age and stock. The two control groups were simultaneously maintained in the B.O.D.

(v) Preparation of stained blood smears

A drop of blood was obtained by cutting a thoracic leg in case of larvae of *D. obliqua* and by puncturing the tip of the head in case of pupa. Whereas, in case of *D. cingulatus*, blood was obtained by cutting the antenna. The drop was put on one end of glass slide, then spread with the help of smooth edge of another slide (held at 45 degree) and with rather quick movement, the spreader slide was pushed towards the other end of the under slide. The thin smear thus formed was air dried. The cells were fixed by covering the smear with a few drops of methyl alcohol for 2-5 min. However, fixation of cells was not necessary for staining with Leishman's stain which already contains a fixative. Before use, Giemsa's stain was mixed with phosphate buffer adjusted to pH 7 in the ratio of 1: 9. The blood film was then covered with the diluted stain for 45 minutes. After that, the slides were rinsed in distilled water and washed thoroughly in running tap water. For Leishman's staining, the air dried smear was covered with staining solution (8-10 drops) for 2 min. Twice number of drops of Leishman's buffer (pH 7) were poured over the slide and kept for 25 min. After that slides were washed in running tap water. All the stained blood smears were dried at room temperature and mounted in DPX.

(vi) Method for Total Haemocyte Count (THC)

Counting of total blood cells was made by means of a haemocytometer with improved double Neubuer ruling as used for counting the white blood cells in human blood. Before drawing blood insects were heat fixed in warm water (55-60°C) for 5 min. Blood was obtained by cutting antenna in case of *Dysdercus cingulatus* and proleg and head, respectively, in case of larva and pupa of *Diacrisia obliqua*. Blood was drawn from *D. cingulatus* up to the mark 0.1 of the pipette used for counting the white blood cells of humans. Then diluting fluid (1.5% glacial acetic acid slightly stained with methylene blue) was also drawn to make the fluid column up to the mark 1 of the pipette. Thus this blood was diluted 9 times. The blood was thoroughly mixed with the diluting fluid by rolling the bulb between the palms of the hands for 5 min. It also facilitated the staining of the nuclei of the haemocytes. Subsequently, after

rejecting the first few drops from pipette, the diluted haemolymph was flown in between the coverslip and Neubuer counting chamber. The chamber was then focused under the microscope and cells were counted in four corner squares. The total number of haemocytes was calculated by the formula (given below) which gave the number of cells per mm^3 of blood volume.

Total number of haemocytes/mm^3 = $\frac{x}{4} \times 10 \times 9$

Where,

x = total number of haemocytes in 4 corner squares of the chamber

4 = Number of corner squares

10= Reverse of the depth of the counting chamber of haemocytometer

$(\frac{1}{10} mm)$

9 = Dilution of the haemolymph

In case of *Diacrisia obliqua* haemolymph was drawn from heat fixed larvae and pupae upto the mark 0.5 of the pipette. Then diluting fluid was drawn so as to make the fluid column upto the mark 11 of the pipette, thus diluting the blood 20 times. Rest of the procedure was same as was followed in case of *D. cingulatus*. The total number of haemocytes was calculated by the formula given below.

Total number of haemocytes/mm^3 = $\frac{x}{4} \times 10 \times 20$

Where,

x = total number of haemocytes in 4 corner squares of the chamber

4 = Number of corner squares

10 = Reverse of the depth of the counting chamber of haemocytometer

$(\frac{1}{10} mm)$

20 = Dilution of the haemolymph

(vii) Method of Differential Haemocyte Count (DHC)

DHC was recorded in permanent stained smears. At least 100 cells were counted and categorized in each smear. One blood smear was made from an individual insect and for each treated and control group five smears were observed. The differential haemocyte count was calculated in terms of percentage of different types of haemocytes in different observations.

(viii) Interpretation of data

The data on THC and DHC of control (solvent treated) and treated *D. cingulatus* and *D. obliqua* were recorded in 5 replicates and mean values of THC and DHC were calculated for each set of experiment. The determination of any change was not only ascertained arithmetically but also appreciated by statistical analysis. It was also necessary to appreciate the difference between mean values of different sets of samples. Arithmetically it was uncertain, therefore, the mean values were tested statistically. For this purpose, the statistical technique of variation significance (t, test) was applied (Bailey, 1959). The calculated `t' was compared with the tabulated 't' at 5% level. If the calculated value remained higher than the tabulated `t' value, the data was significant, otherwise insignificant.

To measure the intensity of association between DHC and concentrations of chemicals, a regression line was fitted to the data followed by correlation analysis (Sockel and Rohlf 1981).

(IX) Transmission Electron Microscopic (TEM) techniques for studying blood cells

The experimental larvae of *Diacrisia obliqua* were chilled at 4°C for about 10 minutes. From such larvae the haemolymph was collected directly by cutting one of

their prolegs in a centrifuge tube containing fixative (2.5% glutaraldehyde in phosphate buffer adjusted to pH 7.2, Sabatini *et al* 1963). The mixture of haemolymph and fixative was centrifuged for half an hour at 3000 rpm. The pellet, thus obtained, was washed thrice with phosphate buffer (7.2 pH) at intervals of half an hour. Finally, the pellet was kept in the buffer overnight. Next day it was post-fixed in 1% osmium tetroxide for 2 hrs, subsequently washed in phosphate buffer (7.2 pH) and distilled water, dehydrated in ascending grades of acetone. Then the pellets were embedded in Araldite-Epon- DDSA mixture. Ultra-thin sections (Silver and Gold) were cut and stained with uranyl acetate and lead citrate as described by Reynolds (1963). The stained sections were examined under TEM (Philips CM 10) and subsequently photomicrographs were taken on Copex pan ahu Pet 10" microfilm.

IV- OBSERVATIONS AND RESULTS

SECTION I

I Observations on free haemocytes of normal *Dysdercus cingulatus.*

The haemocytes of *Dysdercus cingulatus* were studied in the blood smear of both 5th instar nymphs and adults. The observations on haemocytes were made just after moulting and then at the intervals of 24 hr. upto the next moulting. These haemocytes were mainly classified on the basis of their shape, size and staining properties. The smears stained with Giemsa and Leishman's stain showed generally the same results.

1. Classification and description of free haemocytes of *Dysdercus cingulatus.*

The free haemocytes of *D. cingulatus* were classified into five categories viz., prohaemocytes, plasmatocytes, granulocytes, oenocytoids and adipohaemocytes. All the five types were consistently present in 5th instar nymphs and the adult males and females. However, their total number and relative percentage varied according to age and stage. The characteristics of each type of cells is as follows.

Prohaemocytes

These were small, generally round, sometimes ovoid or pear shaped cells (Plate I, Fig. A to E and Plate III, Fig. A to D). The diameter of these cells varied from 5.8 µ to 11.6 µ. The ratio of cytoplasm to nucleus was low. The nucleus occupied almost entire cell leaving only scanty cytoplasm on the periphery which

was usually homogeneous. The cell membrane is smooth, regular and distinct. The nucleus is uniformly packed with fine dot like granules which became more prominent at prophase stage of mitotic cell division. However, the mitotic cell division was mostly seen in larger prohaemocytes. At metaphase stage the chromosomes were so tightly packed that they appeared like a single bar.

Plasmatocytes

These cells were highly polymorphic, assuming various shapes including round, ovoid, pear and spindle shape and occasionally irregular forms (Plate I, Fig. A ,B & D to I; Plate III, Fig. A to D). These cells were generally larger than the prohaemocytes. The size of spherical or ovoid cells varied between 7.6 μ and 25.2 μ, but the spindle shaped cells were 6.2 μ to 9.8 μ in width and 20.0 μ to 46.4 μ in length. The cytoplasm was relatively abundant and sometimes contained a few vacuoles. Nucleus of these cells was comparatively smaller than that of the prohaemocytes, which might be round, ovoid or elongated and central or slightly eccentric in position. These cells were comparatively more numerous and easily distinguishable. Mitosis was frequently seen in these cells and all stages of mitosis i.e. early, mid and late prophase, metaphase, anaphase, telophase and cytokinesis were easily identifiable (Plate III, Fig. F to N). Cells with prominent dark dot like chromatin granules in the nucleus were frequently seen.

Adipohaemocytes

These were round or ovoid cells with spherical, bright fat like inclusions or globules of various sizes (Plate II, Fig. B to I). In young or immature cells the fat droplets were few and the size of cells was relatively smaller In these young cells the cytoplasm was abundant and appeared homogeneous, moreover, the nucleus was distinct. In fully mature adipohaemocytes, the fat droplets completely filled the cytoplasm and sometimes nucleus, too, became eclipsed or partly visible. Various gradations between young and fully mature cells were encountered within the same smear. The nucleus was round or ovoid in young cells containing fine, indistinct nuclear material as well as highly prominent dot like granules. Its position was

usually eccentric, occasionally pushed towards the periphery of cells and sometime central. Binucleate forms were also found. Large fully mature adipohaemocytes with centrally placed nucleus closely resembled fat cells. These cells were generally fragile in nature and were occasionally damaged during smear making and thus leaving only intact nuclei. Mitosis was very rare in these cells and though, very early prophase (i.e. cells showing slight thickening of chromosomes) was some times visible, other stages of mitosis were never encountered in the smear. The size of adipohaemocytes varied between 18.6μ to 50.4 μ.

Oenocytoids

The shape of these cells was generally round and ovoid. The size was larger than prohaemocytes and round plasmatocytes (Plate I, Fig. D & H; Plate III, Fig. A & B). The cell membrane was distinct and regular. The cytoplasm was very smooth, darkly stained and homogeneous. In some cells 1-2 dot like black structures were present near periphery. Occasionally, one or two vacuoles might be present. The nucleus was sharply outlined, relatively smaller than that of prohaemocytes and plasmatocytes, lightly stained, full of dot like chromatin material and was characteristically eccentric. Some times a clear distinction between the nucleus and cytoplasm was difficult. The diameter of these cells ranged between 14.6 μ and 28.4 μ. The population of oenocytoids was very low in the smears as compared to prohaemocytes and plasmatocytes.

Granulocytes

These are usually round, ovoid, and elongated; sometimes spindle shaped but rarely irregular in form (Plate I, Fig. A & I; Plate II, Fig. A). These cells were larger than prohaemocytes. Their size varied between 8.2 μ and 20.6 μ. The cell membrane was distinct and regular. The cytoplasm was relatively abundant and nucleus was small as in plasmatocytes. The cytoplasm was generally filled with numerous discrete, granular inclusions which were sometimes large, light in color and few in number.

The blood smear of one particular nymph was found to contain numerous flagellates characterized by elongated spindle shaped bodies and presence of small oval nuclei as well as a flagellum on one end of the body (Plate III, Fig. O to Q). The flagellum appeared to originate from a small dark dot like body in the proximity of nucleus. The presence of these flagellates in the haemolymph was presumably due to chance infection and was not recorded from any other smears in the present study. No attempt was made to further identify this flagellate.

2. Total Haemocyte Counts (THCs) of free haemocytes of *Dysdercus cingulatus*

In the 5th instar nymphs the THCs have been determined in 1, 2, 4 and 6 days old nymphs. In one day old nymphs THC varied between 2632.5 - 8077.5 cells/ mm^3 blood with a mean value of 4557.0 ± 211.61 cells/ mm^3. In 2 days old nymphs, mean THC ± SE was 6674.5 ± 225.5 cells/ mm^3 (range: 4320-9270 cells/ mm^3). The mean THC was 9499.5 ± 284.99 per mm^3 showing a minimum of 5422.5 cells and maximum of 11520 cells/ mm^3 in 4 day old nymphs. Whereas, in 6 days old nymphs THC was 6212.25 ± 188.30 per mm^3 (range: 4387.5 - 9045 cells).

After 6 days 5th instar nymphs start moulting to adult stage. In the adult stage THC was recorded in 1 day old males and females. Mean THC of 1 day old males was found to be 3630 ± 193.07 cells/ mm^3 varying between 2070 cells - 5602.5 cells/ mm^3. The mean THC of 1 day old females was 6273.75 ± 200.67 (range: 4522.5 - 8032.5 cells / mm^3). In the newly moulted nymphs THC was relatively low, it then steadily increased till 4th day when the peak of THC occurred, followed by a steady decline. The THC in 1 day old females was higher than the males of corresponding age and stage.

3. Differential Haemocyte Counts (DHCs) of free haemocytes of *Dysdercus cingulatus*

To estimate the relative proportions of different types of haemocytes DHCs were determined in the 5th instar nymphs and adults (age-wise as mentioned in case of THC). The prohaemocytes, plasmatocytes, granulocytes, oenocytoids and adipohaemocytes are the main haemocytes of both the nymphs and the adults of *D. cingulatus*. The percentage of these cells has been calculated on the basis of their respective numbers in total number of cells counted in the selected area as described in "Material and Methods". Besides, in every smear there were certain cells which could not be classified into certain type. The percentage of these cells was also calculated. As in the case of THC, the DHC of individuals of each age group vary considerably.

The mean percentage of prohaemocytes in newly moulted 5th instar nymphs was 10.40 which progressively increased to 15.57 on the 4th day. After that the population of these cells in 6 day old nymphs fell to 6.15%. In adult males the mean percentage of prohaemocytes was 3.06 and in adult females it was 5.37. The relative percentage of plasmatocytes in newly moulted 5th instar nymphs was 50.66 and by 4th day it fell to 34.39%. In adult males and females it was 39.41% and 51.08 % respectively.

The population of adipohaemocytes increased from 18.28% in 1 day old to 35.36% in 6 days old nymphs. In one day old adult males the population of adipohaemocytes was maximum (43.92 %) and in adult females it was 27.01%. The population of adipohaemocytes fluctuates inconsistently. In one day old nymphs granulocytes were 11.22%, in 2 day their percentage dropped to 8.66 and then at 4th day it increased to 10.40. Furthermore, in the full grown, 6 day old, 5th instar nymphs it was 12.05%. In one day old adult males and females the granulocytes were 6.82 % and 8.24% respectively.

In the nymphs as well as in adults, the oenocytoids were poorly represented in comparison to prohaemocytes, plasmatocytes and adipohaemocytes. The

percentage of oenocytoids was slightly higher in nymphs than in adults. The highest population (4.28%) of these haemocytes occurred in 6 day old nymphs. The percentage of cells undergoing mitosis fluctuated inconsistently. In one day old nymphs their percentage was 2.25, in 2 day old it was 1.90, in 4 day old nymphs population increased to 2.87 and by 6^{th} day it was found to be 2.30. In adult males and females the mitosis was observed in 2.61% and 2.35% cells, respectively. Besides, above mentioned categories, on an average, 4-6% cells in each smear were either damaged or could not be identified properly.

II Observations on free haemocytes of *Dysdercus cingulatus* treated with different chemicals

Four concentrations, ranging from Lc-30 to Lc-90 in case of acephate aminocarb and cypermethrin and from Lc-20 to Lc-60 in case of muristerone and methoprene, were applied topically to 1 day old 5^{th} instar nymphs. The mortality, moulting and haemocyte picture were subsequently observed 6 hrs after treatment, after 1 day, 3 days and 5 days and finally in 1 day old adult males and females.

1. Effect of topical application of different concentrations of Acephate (an organophosphate) on *Dysdercus cingulatus*

Insects affected with the lowest concentration showed abnormal movements. Within 24 hrs of application 30% mortality occurred. Thereafter general condition of the treated nymphs did not deteriorate further and they showed recovery from toxic effects. Feeding was not affected. Nymphs affected by 0.002% concentration showed occasional signs of irritation in their month parts and legs. Insects showed sluggishness and no inclination towards food. Within 24 hrs 45-50% insects died. Some of the survived nymphs showed occasional vomiting of colourless fluid. Further changes in external symptoms did not occur. Within 2 days all the survived insects showed recovery.

The nymphs affected with 0.004% concentration showed rapid movements of the legs just after treatment and tried to wipe the drop of insecticide from their thorax. Within 24 hrs of treatment the nymphs were paralyzed. Feeding was inhibited during first two days. Approximately 65-70% nymphs died in 24 hrs. After that period some insects showed recovery and resumed feeding. When the treated nymphs underwent imaginal ecdysis, about 5-10% suffered mortality. In the successfully moulted adults feeding was normal, however, the size of these adults was comparatively smaller than the control.

Following the application of the highest concentration (0.006%) of acephate the external pathological symptoms were more severe. Vomiting was very copious and occurred with irritation in mouth-parts. Within an hour of treatment paralysis was also seen. All the insects were found upside down on the bottom of jar. However, some insects still kept on twitching their legs. Moreover, the regurgitation of yellow coloured liquid continued in paralyzed insects. Heavy mortality (approximately 80-85%) occurred within 24 hrs of application. Only a very small percentage of the paralyzed insects survived beyond 72 hrs. However, in these survived insects some blood was available for observations even after 5 days, but none of the insects survived to reach adult stage.

1.1 Haemocyte picture of 5th instar nymphs and adults of *Dysdercus cingulatus* affected with different concentrations of Acephate.

After 6 hrs of application of 0.001% and 0.002% acephate most of the haemocytes appeared like those of normal insects except a few adipohaemocytes which showed presence of small vacuoles in their cytoplasm (Plate-IV, Fig. A). The 0.004% concentration induced abnormal vacuolization in the cytoplasm of more adipohaemocytes and some plasmatocytes. The prohaemocytes, granulocytes and oenocytoids were mostly like those of normal. The nucleus of many haemocytes exhibited dark and prominent chromatin material as found in very early prophase. The highest concentration caused more vacuolization in cytoplasm, irregular cell

membrane occasionally broken at some places, and appearance of many small vacuoles in the nucleus of plasmatocytes as well as in granulocytes. The adipohaemocytes showed most pronounced pathological symptoms. However, oenocytoids and prohaemocytes were not largely affected. Mitosis was observed even in the damaged cells (Plate-IV, Fig. H).

One day following the treatment more intense histopathological changes occurred in the haemocytes. The adipohaemocytes and plasmatocytes were affected even by the lower concentrations (0.001 & 0.002% acephate). There was precipitation of chromatin material of the nuclei, whereas, the cytoplasm was highly vacuolated especially in plasmatocytes (Plate-IV, Fig. C). By 0.004% acephate, plasma became thick in consistency and clumping of haemocytes was observed in smears. By the highest concentration the adipohaemocytes were generally not visible, however, fragments of their cytoplasmic portions along-with damaged nucleic were present in the smears. Majority of the other haemocytes showed abnormalities in cytoplasm as well as in nucleus. Furthermore, nearly half of the total cell population was damaged beyond recognition. In these cells severe nuclear and cytoplasmic disintegration occurred. Amongst the cells which could be identified, the plasmatocytes showed abnormal cell shape and size, vacuolated cytoplasm having small and large sized vacuoles which probably represent loss of material from the cell, abnormal cytoplasm extensions and in some cases outflow of cytoplasmic material (Plate-IV, Fig. B & J). The oenocytoids had swollen nucleus, damaged cell membrane and cytoplasmic discharge. However, vacuolization was not intense in these cells.

Three days after treatment with the lowest concentration, all the haemocytes were like those of normal except adipohaemocytes which exhibited vacuolated cytoplasm. No other noticeable changes occurred in other haemocytes. In the smears affected with 0.002 and 0.004% concentrations the cell changes, in general, included agglutination, much distortion and some disintegration. However, different cell types differed in their reactions to the poison. Adipohaemocytes were mostly disintegrated and the remaining few adipohaemocytes were with large vacuoles. Plasmatocytes showed tendency to swell and a few of them became somewhat

achromophilic as well as their cytoplasm became excessively vacuolated and the nuclear membrane became ragged (Plate-IV, Fig. L). Nuclear distortion, disintegration and extrusion was also noticed. The prohaemocytes were comparatively intact. However, irregular ragged cell membrane, vacuolation of nucleus and flow of cellular contents into surroundings occurred. The few oenocytoids, that were noted, appeared to be unaffected. With the highest concentration, majority of cells showed damage and blood smear mostly indicated cell debris and fragments of nuclei of degenerating cells. The adipohaemocytes and granulocytes were completely destroyed leaving only a few nuclei which were generally clumped. Prohaemocytes were also highly vacuolated. But most of them were intact and recognizable.

Subsequent to the treatment with lower concentrations almost all haemocytes recovered after 5 days of application. However, 0.004% concentration of acephate destroyed all the adipohaemocytes and granulocytes. The plasmatocytes showed cytoplasmic bulgings, extensions or pseudopodial structures, whereas, some of these cells indicated outflow of cytoplasmic contents. The differentiation between cell and nuclear material was difficult because of discharge of nuclear contents into the cytoplasm (Plate-IV, Fig. P). Mitosis was observed even in the damaged cells. The prohaemocytes were with vacuolated cytoplasm, irregular cell membrane and vacuolated nuclear contents (Plate-IV, Fig. K). The oenocytoids were least affected showing only a few vacuoles and sometime damaged cell membrane. Following treatment with the highest concentration of acephate (0.006%) blood yield was very low. About half of the haemocytes were damaged beyond recognition (Plate-IV, Fig. M, N & O). The remaining cells exhibited varying degrees of distortion and damage. The major population of haemocytes comprised prohaemocytes and damaged plasmatocytes (Plate-IV, Fig. E & G). Since the cytoplasmic content of prohaemocytes is scanty, these cells mostly showed vacuolated nuclei, shrinkage in nuclear membrane and gross irregularities in the nuclear shape. The size of nucleus also became apparently reduced due to disintegration of nuclear material and discharge of nuclear contents.

The plasmatocytes exhibited enormous number of cells having grossly abnormal cytoplasmic and nuclear changes as well as abnormal changes in shape, volume and distortion of configuration of these haemocytes. The nuclear changes were expressed as gross irregularities in shape, nuclear swelling (hypertrophy), pycnosis (atrophy), nuclear vacuolation and rupturing (karyorrhexis) (Plate-IV, Fig. D & F) as described in other insects. The cytoplasmic changes included abnormal vacuolation, staining reaction, rupturing of cell membrane and discharge of cytoplasmic contents. While making differential haemocyte counts these abnormal cells were grouped under damaged cell category. The adipohaemocytes were completely damaged. The granulocytes and the plasmatocytes with induced granulation could not be distinguished from each other. Although most of the haemocytes were severely affected, a few prohaemocytes, plasmatocytes and oenocytoids appeared just like those in the normal blood. Probably these cells were differentiated after the treatment and were freshly released into the circulation. The other possibility being the recovery of these cells from toxic effects of poison. All the remaining survived nymphs, which were maintained to moult to adult stage and their haemolymph was not yet withdrawn, eventually died, consequently no observations could be made on the adult haemolymph affected with 0.006% concentration of acephate.

The haemocyte picture of affected adult males and females emerged from treated 5th instar nymphs with 0.001, 0.002 and 0.004% acephate did not deviate much from the normal solvent treated control of the corresponding age. Occasional vacuoles in cytoplasm and nuclei of plasmatocytes and adipohaemocytes were noticed. Prohaemocytes of 0.004% acephate affected males and females appeared like those of normal adults. However, some were smaller in size. Many intermediate forms of haemocytes were also present which could not be placed in any definite category

1.2 THCs of 5th instar nymphs and adults of *Dysdercus cingulatus* affected with different concentrations of Acephate

THCs of affected nymphs by the 0.001, 0.002, 0.004 and 0.006% concentrations were taken at 6 hrs. 1 day, 3 days and 5 days after treatment with respective concentrations. These are summarized in Table 1. When the treated nymphs moulted to adult stage, counts were made in 1 day old males and females, respectively.

After 6 hrs

In the nymphs of untreated and control stock of the respective age, the THCs were 4630 ± 562.74 and 4536 ± 622.02 cells/mm^3. By the lowest concentration there was a slight increase (3.67%) in cell count. Following application of 0.002%, 0.004% and 0.006% acephate, THC was reduced by 9.33%, 38.69% and 43.55% which were statistically insignificant ($P>0.05$).

After 1 day

In the untreated and acetone treated nymphs (control) the respective THCs of the given stage were 6457.5 ± 206.07 and 6592.5 ± 647.71 cells/mm^3 of haemolymph. By the application of the lowest concentrations (0.001%) the THC was increased by 4.44% which was statistically insignificant. Following the treatment with higher concentrations (0.002, 0.004 and 0.006%) the THCs were dropped by 18.03%,41.02% and 43.56% respectively which were also statistically insignificant compared to the control.

After 3 days

The THCs of untreated and solvent treated 5th instar nymphs were 9274.5 ± 527.50 and 9180 ± 438.09 respectively. After 3 days of treatment with 0.001% concentration there was a slight reduction in the THC (9.07%). However, 0.002%

concentration caused destruction of about ¼th of cell population which was, however, statistically insignificant at 5% level. The higher concentrations (0.004 and 0.006% acephate) resulted in 58.82% and 67.40% reduction in THCs which was highly significant (t=3.8016 and t=4.0476, P<0.05).

After 5 days

The normal and acetone treated nymphs at this stage exhibited total cell counts as 6435.0 ± 356.32 and 6669.0 ± 684.30, respectively. The THCs of nymphs affected by two lower concentrations (0.001 and 0.002%) were reduced by 8.23% and 20.58%, respectively. The 0.004% concentration caused a reduction of 39.44% which was considerably less than that in THC at 3 day post treatment. However, after 5 days following 0.006% acephate, the fall in THC was by 67.54% compared to control which was almost similar to that found in nymphs after 3 days of treatment (67.40% t=3.1498, P<0.05).

After imaginal ecdysis

The THCs of 1 day old untreated males were 3528.0 ± 389.99 whereas that of males emerged from acetone treated 5th instar nymphs (control) were 3532.5 ± 413.47. There was almost negligible reduction (1.66%) in THC with respect to the 0.001%. The THC of adult males emerged from 0.002% treated nymphs was 15.29% less. Furthermore, the 0.004% concentration caused a reduction of 26.50% as compared to control.

One day old normal females had 6372.0 ± 527.74 cells/mm^3 of blood. The acetone treated (control) females showed THC as 6448.5 ± 745.38 cells/mm^3. In the females emerged from 5th instar nymphs treated with 0.002 and 0.004% concentrations of acephate reductions in THCs were by 18.98% and 30.21% as compared to control. Following the application of 0.006% acephate, 100% mortality occurred within 5th instar stage, therefore the THC could not be determined in adult stage.

1.3 DHCs of 5th instar nymphs and adults of *Dysdercus cingulatus* affected with different concentrations of Acephate.

The relative percentage of prohaemocytes, plasmatocytes, adipohaemocytes, granulocytes and oenocytoids of affected nymphs were determined 6 hrs, 1 day, 3 days and 5 days following the treatment with all the selected concentrations of acephate. Moreover, the relative population of damaged/unidentified cells was also calculated. When the treated nymphs moulted to adult stage, DHC was ascertained in one day old adult males and females. The DHCs of treated nymphs and affected adults were compared with those of the control insects of corresponding age and stage.

After 6 hrs (Table-6, Fig.1)

Six hrs. after application of acephate the percentage of prohaemocytes exhibited a linear increase with increase in concentration ($Y=12.37 + 2636.89 X$, $r = 0.9399$, $P<0.001$) showing a positive correlation coefficient. With the highest concentration (0.006%) the population of prohaemocytes became nearly three times of the control. The plasmatocytes constituted 53.2% of haemocytes in control nymphs, which subsequently exhibited a negative linear correlation with increase in concentration of acephate applied ($Y = 48.80 - 6741.72 X$, $r = -0.9779$, $P<0.001$). The adipohaemocytes in the normal and acetone treated nymphs of corresponding age constituted 16.58% and 17.06% of total haemocytes. Following the application of graded concentrations (0.001, 0.002 and 0.004%) their relative occurrence was 15.84%, 11.32% and 9.72% respectively. However, in the nymphs affected with the highest concentration (0.006%) these haemocytes were either completely absent or completely damaged showing only the remains of cytoplasm and nuclei in the smear The density of granulocytes in smears obtained from nymphs, which were affected with 0.001, 0.002 and 0.004% acephate, decreased linearly with increase in concentrations applied ($Y =10.57 - 827.24 X$, $r = -0.9884$, $P<0.001$). The oenocytoids were relatively few in number in normal and acetone treated insects. In control, the mean percentage of oenocytoids was 3.15 which increased linearly with increase in concentration of acephate applied ($Y =3.87 + 1707.07 X$, $r = 0.9595$, $P<0.001$)and

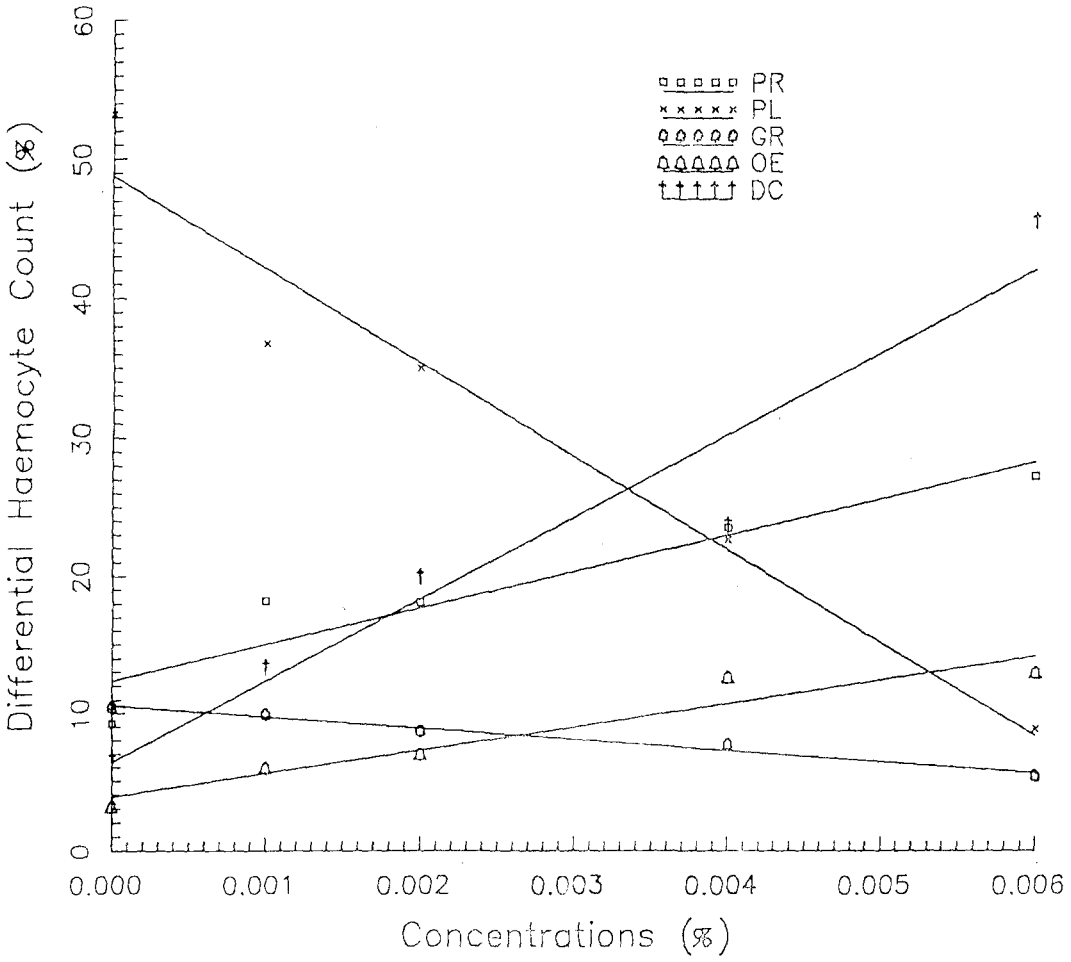

Fig. 1: Correlation between the Differential Haemocyte Count (%) and various concentrations of acephate after 6 hrs. of the treatment on one day old 5th instar nymphs of Dysdercus cingulatus.

reached to 12.9% with the highest concentration. Even though their number in the smears did not alter much, their relative percentage showed about four and a half times increase which might have been due to the fact that most of these cells did not exhibit severe damage as was found in case of plasmatocytes and adipohaemocytes. Besides, all those cells which could not be identified either due to distortion caused during smear making or due to abnormalities caused by the action of insecticides were also included. Consequently, the application of 0.001, 0.002, 0.004 and 0.006% acephate resulted in the appearance of respectively 13.34, 19.92, 23.72, and 45.62% damaged cells after 6 hrs of application exhibiting a positive linear correlation with increasing concentration of acephate as compared to control which showed 6.64% abnormal cells.

After 1 day (Table-7, Fig.2)

The density of prohaemocytes progressively and linearly increased with increasing concentrations ($Y = 17.0529 + 2358.10 X$, $r = 0.8385$, $P<0.001$) showing 27.34% ($t=2.268$, $P>0.05$) cells in 0.004% acephate affected smears compared to 11.96% in control. However, by 0.006% acephate there was slight increase in their number compared to 0.004% concentration. The plasmatocytes were highly affected cells. Their population in control and untreated insects was 43.62% ± 3.16 and 47.12% respectively. There was a concentration based linear reduction ($Y = 34.73 - 4977.41 X$, $r = - 0.8847$, $P<0.001$) in the density of these cells showing a negative correlation coefficient. The highest concentration reduced the population of these cells to 7.26% showing a statistically significant reduction ($t=4.566$, $P<0.05$) compared to control. Similarly, the adipohaemocytes showed an inverse linear relation to the increasing doses of acephate ($Y = 22.99 - 4252.41 X$, $r = - 0.9604$, $P<0.001$). Furthermore, following treatment of the highest concentration the adipohaemocytes disappeared from the smears. The population of granulocytes increased to 15.32% and 9.14% following treatment with 0.001 and 0.002% acephate as compared to 8.42% in the control. However, by the higher concentrations (0.004 and 0.006%) the population of granulocytes was reduced to 5.62% and 1.74% compared to control (8.42%). Subsequent to the application of 0.001, 0.002, 0.004 and 0.006% concentrations the relative percentage of oenocytoids was progressively increased

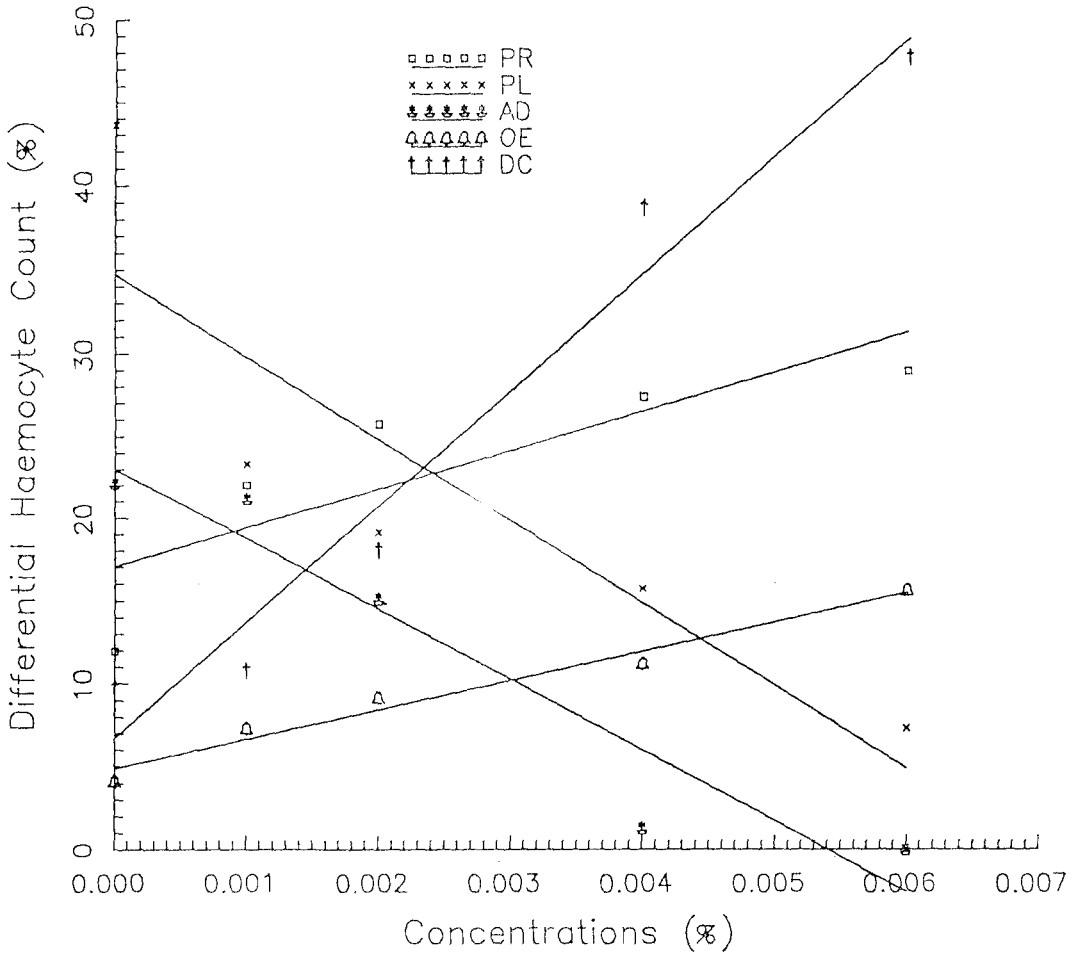

Fig. 2 : Correlation between the Differential Haemocyte Count (%) and various concentrations of acephate after one day of the treatment on one day old 5th instar nymphs of Dysdercus cingulatus.

linearly (Y = 4.897 + 1756.38 X, r = 0.9855, P<0.001), however, only a few oenocytoids showed damage and most of these cells were recognizable even in the smears of 0.006% acephate affected nymphs. The proportion of damaged and disintegrated cells was enhanced with increase in concentration of insecticide applied showing a positive linear correlation (Y = 6.716 + 7002.93 X, r = 0.9822, P<0.001). The highest concentration caused destruction of 47.56% haemocytes compared to 9.72% in control showing a statistically significant increase (t= 4.174, P<0.05).

After 3 days (Table-8, Fig.3)

Three days following treatment with 0.001 and 0.002% acephate the percentage of prohaemocytes exhibited an increasing trend. However, with 0.004 and 0.006% concentrations there was slight fall in their population compared to lower concentrations. In the smears of control nymphs, plasmatocytes constituted 33.78% of total cell population. Subsequent to the application of 0.001, 0.002, 0.004 and 0.006% concentration, the respective percentage of these haemocytes was 24.58, 14.34, 13.0 and 10.7 showing concentration based linear reduction (Y = 28.378 - 3499.14 X, r = -0.8694, P<0.001) with increasing concentrations of acephate which was statistically significant at 0.004 and 0.06% concentration (t=2.891 and t=3.207 respectively, P<0.05). The adipohaemocytes were most fragile cells and were highly damaged even by lower concentrations. Following 0.002% acephate the population of adipohaemocytes was reduced to one third. Moreover, by 0.004 and 0.006% acephate the adipohaemocytes were completely damaged. similarly, the population of granulocytes progressively increased to nearly double by 0.004% concentration. However, following the highest concentration these cells were not found in the smear. Oenocytoids were the least affected cells in the smear. Although, the number of these cells remained fairly constant in the treated insects their relative percentage showed an increase due to disintegration of other types of cells. Following the highest concentration of acephate the population of these cells increased to 17.5% compared to 3.72% in control showing a concentration based linear increase with increase in concentrations (Y = 4.256 + 2410.69 X, r = 0.9796, P<0.001).

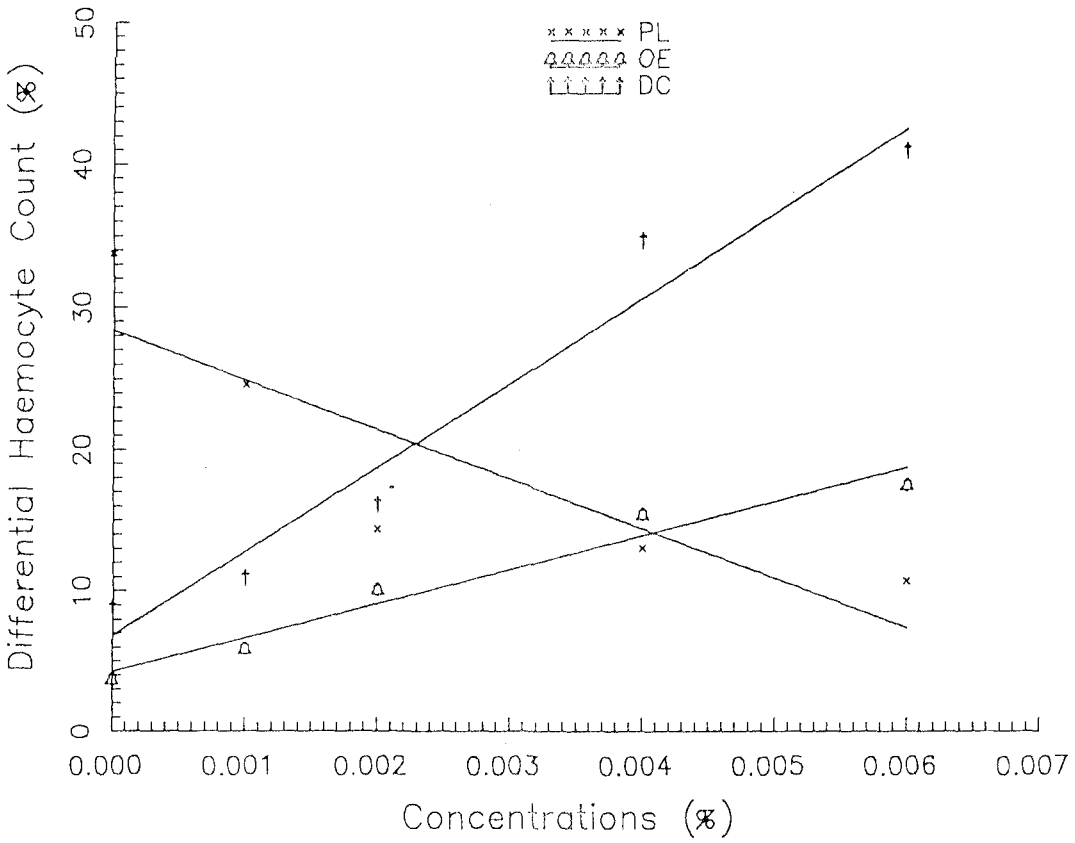

Fig. 3 : Correlation between the Differential Haemocyte Count (%) and various concentrations of acephate after 3 days of the treatment on one day old 5th instar nymphs of Dysdercus cingulatus.

There was a consistent increase in the proportion of damaged/unidentified cells in accordance with the increase in concentration of acephate applied (Y= 6.849 + 5932.07 X, r = 0.9803, P<0.001). Thus a maximum of 40.9% (t=3.889, P<0.05) cells were found damaged/disintegrated with the highest concentration of acephate showing statistically significant increase.

After 5 days (Table-9, Fig.4)

After 5 days of the treatment with different concentrations of acephate prohaemocytes showed an increase in their population, highest being in 0.004% acephate treated nymphs. Plasmatocytes were severely affected by 0.004 and 0.006% concentrations comprising only 14.10% and 02.62% of total cells showing statistically significant (t=3.655 and t=4.070, respectively, P<0.05) reduction in their population and having a negative linear correlation (Y = 33.034 - 5071.72 X, r = -0.9842, P<0.001) with increasing concentrations of acephate compared to control (35.82%). Adipohaemocytes were significantly less (19.52%) in 0.002% acephate affected smears (t=3.014, P<0.05), however, these cells were completely damaged by 0.004 and 0.006% acephate. Similarly, granulocytes, too, were unidentifiable in smears affected with higher concentrations. The population of oenocytoids showed a steady increase with the increase in concentration of acephate displaying a positive linear correlation (Y = 4.537 + 2136.38 X, r = 0.9813, P<0.001). Their relative percentage was almost six and a half times higher in 0.004 and 0.006% acephate treated nymphs. After five days following application of 0.006% acephate, 47.08% cells were unidentifiable and with 0.004% concentration there were 41.92% cells unidentifiable compared to 6.82% in control thereby showing a statistically significant reduction (t=3.192 and t=4.061, P<0.05) and exhibiting a positive correlation (Y= 3.851 + 7731.21 X, r = 0.9673 P<0.001) with increasing concentrations. On the other hand, with two lower concentrations, the effect on population was statistically insignificant.

After imaginal ecdysis

When the treated nymphs moulted to adult stage, the relative percentage of each type of haemocytes was calculated in males and females separately.

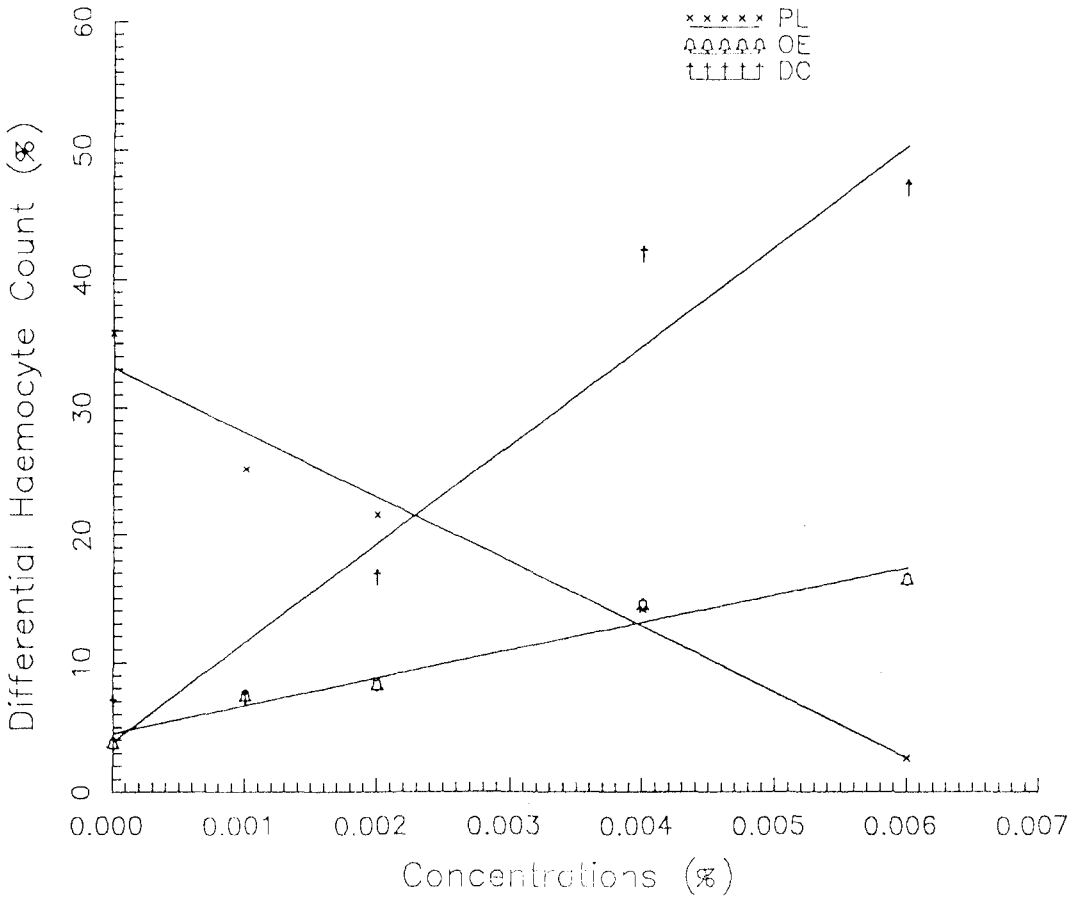

Fig. 4 : Correlation between the Differential Haemocyte Count (%) and various concentrations of acephate after 5 days of the treatment on one day old 5th instar nymphs of Dysdercus cingulatus.

In one day old males (Table-10), the prohaemocyte population showed statistically insignificant increase with the application of lower concentrations. However with 0.004% acephate there were approximately five times more cells in the smear compared to control. Plasmatocyte population inconsistently decreased showing statistically insignificant reduction. Similarly, adipohaemocytes showed insignificant reduction by the application of different concentrations of acephate. Granulocytes and oenocytoids too followed the same trend. Likewise, population of damaged cells showed statistically insignificant change.

In the females emerged from treated nymphs (Table-11) only prohaemocytes showed statistically significant increase by 0.004% acephate ($t=3.089$, $p<0.05$) compared to control. Besides that all the other haemocyte types exhibited inconsistent and statistically insignificant change.

2. Effect of topical application of different concentrations of Aminocarb (a carbamate) on *Dysdercus cingulatus*

With the application of different concentrations of aminocarb, insects developed pharmacological and external symptoms depending on the gradation of the chemical applied. Since the concentrations of insecticides were selected on the basis of mortality resulting in first 24 hrs, the aminocarb produced almost similar mortalities as caused by the application of corresponding concentrations of acephate. For example the lowest concentration of aminocarb (0.0025%), like that of acephate, induced approximately 30% mortality, which increased to 50 and 70% when subjected to 0.004 and 0.007% aminocarb respectively and the highest concentration (0.008%) being the most fatal perished about 85% insects in a day. Treatment with 0.0025% aminocarb induced sluggish movements in nymphs after about 1 hr of its application. Some nymphs started vomiting a colorless fluid and occasionally a yellowish fluid was discharged through anus. In certain nymphs effects were severe, however, after 1 day all the survived insects showed recovery from the pharmacological symptoms and feeding was not affected.

Nymphs affected by 0.004% aminocarb initially displayed rapid movements of their legs and ran haphazardly in the jar. Paralytic effects were viewed after 30-45 minutes, followed by faulty orientation of body, twitching of legs and antennae. And within 2-3 hrs about 70% insects were found lying on their dorsum at the bottom of the jar. About 45-50% nymphs were found dead after 1 day following application, however, remaining insects recovered from toxic effects. In addition to that about 5-10% mortality occurred during the imaginal ecdysis. Furthermore, the successfully emerged adults were free from any pharmacological effects. But certain adults showed morphological deformities such as development of malformed wings and legs. The nymphs treated with 0.007% aminocarb showed more severe external symptoms as described with respect to other concentrations. Paralysis of about 90% of insects occurred within 3-4 hrs and they were found on their back in the bottom of the jar. However, the paralysis was not complete and the nymphs kept on twitching the legs. On the other hand, about 10% of the nymphs still showed normal movements and were occasionally found sitting on petri-dishes containing food. After 1 day about 60-65% treated nymphs suffered mortality and the survived insects showed partial recovery evident by their slow movements and occasional feeding. Mortality continued to occur in 1-2% nymphs daily. About 15-20% of total treated nymphs successfully moulted to adult stage. Feeding was resumed in recovered nymphs and adults. The external symptoms of nymphs treated with the highest concentration (0.008%) were most severe. All the treated nymphs were found on their dorsum within half an hour of the treatment. There was very copious vomiting, moreover, yellowish watery discharge through the anus was preceded and followed by paralysis. Twitching of legs was occasionally seen and there was no feeding response. After 1 day 80-85% insects were found dead. In the survived insects there was partial recovery from paralysis. One or two nymphs suffered mortality daily, up to 5 days and the remaining died at the time of moulting. Therefore, no adults emerged from the nymphs affected with this concentration. Moreover, the haemolymph of the treated nymphs became viscous and yield was low. Therefore, sometimes, blood of 2-3 nymphs was pooled to make smear and for the estimation of THC.

2.1 Haemocyte picture of 5th instar nymphs and adults of *Dysdercus cingulatus* affected with different concentrations of Aminocarb.

After 6 hrs. of treatment with the 0.0025 and 0.004% aminocarb, the haemocytes did not show any remarkable alteration in their normal morphology except occasional vacuoles in plasmatocytes and adipohaemocytes and granules in cytoplasm of some cells (Plate-V, Fig. B). With the 0.007% aminocarb most of the spindle shaped plasmatocytes became round and showed vacuolation in cytoplasm. Prohaemocytes remained unaffected (Plate-V, Fig. A). Mitosis was observed in more cells compared to control. Following the application of the highest concentration (0.008%), plasmatocytes showed intense vacuolation in their cytoplasm. Similarly, granulocytes also developed vacuoles in cytoplasm as well as in nucleus. Adipohaemocytes were most severely affected, leaving only nuclei in the smear. However, a few of them could still be recognized, which contained large vacuoles. Furthermore, fat droplets, which were abundant in normal adipohaemocytes, also seemed to disappear from these haemocytes (Plate-V, Fig. G). Oenocytoids had less distinct cell membrane and showed slight discharge of cytoplasm. Mitosis was observed even in some severely affected haemocytes which had already lost their identity. In addition to the above mentioned effects, a large number of haemocytes were affected in such a way that their morphological details became obscure and the differentiation of cytoplasm and nuclei became less distinct due to poor staining of the cells (Plate-V, Fig. D).

After 1 day of treatment, the plasmatocytes and adipohaemocytes were worst affected cells (Plate-V, Fig. C). They exhibited almost similar pathological effects as found with the corresponding concentrations of acephate. Some plasmatocytes, however, showed only slight damage. Oenocytoids were identifiable even after the application of the highest concentration (Plate-V, Fig. F). However, this concentration completely destroyed adipohaemocytes and made granulocytes unrecognizable due to intense vacuolization of cytoplasm. The nuclei of most of the plasmatocytes and some prohaemocytes showed vacuolization, precipitation and

clumping of chromatin material. Besides these effects, clumping of haemocytes was occasionally observed. Moreover, plasma exhibited numerous folds in the smear.

In the blood smears 3 days after the treatment with lower concentrations, the adipohaemocytes showed appearance of large vacuoles in cytoplasm and discharge of fat like droplets from these cells into the plasma (Plate-V, Fig. E). With high concentrations (0.007 and 0.008%) these cells were absent from the smear. The plasmatocytes affected with lower concentrations showed formation of small vacuoles in cytoplasm, broken cell membrane and discharge of cytoplasm from the cells. However, following application 0.007% aminocarb more plasmatocytes had pathological symptoms (Plate-V, Fig. H). Mitosis was not inhibited even in the severely affected plasmatocytes. 0.0025 and 0.004% aminocarb caused granulation in cytoplasm of more haemocytes. Moreover with 0.007% aminocarb the granulocytes developed small vacuoles in cytoplasm and ragged cell membrane but most of these cells were still distinguishable. However, these cells became unrecognizable in the smears affected with the highest concentration (0.008%). The cytoplasmic changes in oenocytoids included apparent swelling, achromophilia and formation of broad pseudopodia or cytoplasmic bulgings, however, these degenerative changes occurred following treatment with the highest concentration only. And even then these haemocytes were easily distinguishable. There was an apparent increase in the mitotic activity which was evident in the nuclei of some cells by assuming a condition suggestive or indicative of the beginning of prophase of mitosis. Other advance stages of mitosis were also observed in blood smears of both aminocarb affected nymphs and control nymphs. Even those haemocytes which showed marked cytological damage sometimes exhibited advanced stages of mitotic cell division. Prohaemocytes, by virtue of having scanty cytoplasm underwent minor cytoplasmic degeneration showing small thread like processes on cell surface, irregularity of cell membrane and few vacuoles. Only a small population of prohaemocytes was affected with 0.0025 and 0.004% aminocarb, whereas, higher concentrations induced similar effects accompanied with disintegration of nuclear material.

Five days following the treatment with 0.0025 and 0.004% aminocarb, haemocytes displayed minor cytoplasmic and nuclear changes and prohaemocytes appeared to be predominant type in such smears. Treatment with 0.007% aminocarb resulted in marked changes which were characterized by the extensive vacuolation of granulocytes and plasmatocytes and complete destruction of adipohaemocytes (Plate-V, Fig. I). The nymphs affected with the highest concentration of aminocarb contained very little blood, probably due to water loss during vomiting and discharge through anus as well as negligible feeding. The haemolymph smear contained about 45% haemocytes showing complete destruction. In addition to that, cell agglutination was considerable and plasma showed many folds due to high viscosity leading to uneven distribution of haemocytes in the smears. The kinds of abnormalities were strikingly numerous and complex in nature (Plate-V, Fig. J, K & L) viz., cells with intensely basophilic cytoplasm or cytoplasm stained very unevenly so as to appear blotched, or with very large nuclei which were either reticulate or vacuolated. The damages in prohaemocytes were less severe, moreover, a fair percentage of these cells and few plasmatocytes appeared absolutely normal, the possible explanation for their apparently normal morphology may be due to fresh supply of haemocytes to haemolymph from haemopoetic organs or through mitotic/ amitotic cell division.

Since present photomicrographs show only small area of the blood smears, they do not represent all the cell types, affected or unaffected, so adequately as is possible by the more extensive microscopic examinations of the entire smear. Furthermore, the cytological details are not visible so clearly in the photomicrographs as they appeared in the smears.

When the treated nymphs moulted to adult stage, the haemolymph smears of the males and the females were observed 1 day post emergence. Blood picture of one day old males affected with 0.0025 and 0.004% aminocarb appeared to be similar to that of control. Moreover, the mitotically dividing cells were numerous in smears of 0.007% aminocarb. Large vacuoles were noticed in some adipohaemocytes. Other abnormalities noted among some plasmatocytes were extremely spread forms, reticular cytoplasm, nuclear swelling and discolouration of

cells etc. Granulocytes and oenocytoids appeared like those of normal. Similarly the females contained a large number of prohaemocytes as evident from corresponding DHCs. Besides that granulation was found in more haemocytes than control. Other changes were same as found in the males.

2.2. THCs of 5th instar nymphs and adults of *Dysdercus cingulatus* affected with different concentrations of Aminocarb.

THC of 5th instar nymphs was recorded 6 hrs, 1 day, 3 days and 5 days following the treatment as well as in 1 day old adult males and females emerged from the treated nymphs and the data is summarized in Table-2.

After 6 hrs

THCs of untreated and solvent treated (control) nymphs of the respective age and stage was 4702.5 ± 499.23 and 4617 ± 520.74 respectively. Following the treatment with 0.0025% aminocarb, there was 12.76% increase in average cells/mm^3 compared to control. However, with 0.004 and 0.007% aminocarb, the THC decreased by 12.67% and 32.16%, respectively. The highest selected concentration (0.008%) within 6 hrs of application caused a fall of 58.28% ($t=3.0653$, $P<0.05$) in cell number per mm^3 of blood.

After 1 day

One day following aminocarb treatment the cell population continued to increase (9.85%) though slightly less than that found after 6 hrs (12.76%). By 0.004% concentration there was 32.04% reduction in THC which further registered a fall of 60.04% and 74.75% ($t=3.4351$ and $t= 3.9635$, respectively, $P<0.05$) following 0.007 and 0.008% aminocarb.

After 3 days

Even the lowest dose caused a reduction of 22.40% in THC 3 days after treatment. By 0.004, 0.007 and 0.008% aminocarb the reduction in THC was very high showing respectively 48.95% ($t=2.5773$, $P>0.05$), 71.92% ($t=4.0336$, $P<0.05$) and 81.68% ($t=4.9097$, $P<0.05$) fall as compared to control. The THCs of control and untreated nymphs were 9679 cells/mm^3 and 10246.5 cells/mm^3 of haemolymph, respectively.

After 5 days

Blood cells showed some regeneration after 5 days, consequently, reduction in average THC/mm^3 was found to be comparatively less than that on 4^{th} day after treatment. In the untreated and control nymphs the respective values of THC s were 6250 ± 334.14 cells/mm^3 and 6502.5 ± 661.97 cells/mm^3 of blood. Compared to control the cell number was reduced by 6.57% and 27.27% in the nymphs treated with 0.0025 and 0.004% aminocarb respectively. Next two higher concentration resulted in reduction of 64.7% and 77.51% ($t=3.3706$ and $t=3.5421$, $P<0.05$) in THCs which were statistically significant at 5% level.

After imaginal moulting

When the treated nymphs moulted to adult stage, the THCs of the male and the female were recorded separately. In the males there were 3791 ± 230.18 cells/mm^3 in untreated insects compared to 3384 ± 112.62 cells/mm^3 in acetone treated insects (control). The fall in THCs by 0.0025, 0.004 and 0.007% concentrations of aminocarb was found to be 6.97%, 18.72% and 41.22%, respectively, compared to control.

The one day old females of normal stock and those of untreated stock respectively had 6106.5 ± 499.66 cells and 5949 ± 288.11 cells per mm^3 of haemolymph. The 0.0025% aminocarb affected females exhibited slight reduction in

THC (7.79%) as compared to control, however, 0.004 and 0.007% aminocarb affected females showed 16.94% and 23.68% fall in average THCs.

2.3 DHCs of 5th instar nymphs and adults of *Dysdercus cingulatus* affected with different concentrations of Aminocarb.

As described earlier, the changes in relative proportions of prohaemocytes, plasmatocytes, oenocytoids adipohaemocytes and granulocytes were determined after 6 hrs, 1 day, 3 days and 5 days of treatment with different concentrations of aminocarb on 5th instar nymphs of *D. cingulatus*. When the treated nymphs moulted to adult stage, the DHCs were calculated in one day old males and females.

After 6 hrs (Table-12, Fig.5)

The prohaemocyte population progressively increased with increase in the aminocarb concentration, however, with the highest concentration there was slight reduction compared to 0.007% aminocarb. On the other hand, a negative linear correlation ($Y = 51.85 - 5193.13 X$, $r = -0.9839$, $P<0.001$) was observed in plasmatocyte population with increasing concentrations. Adipohaemocytes were also highly affected haemocyte type undergoing steady decline ($Y = 18.909 - 2087.57 X$, $r = - 0.9831$, $P<0.001$). Granulocytes varied inconsistently in relation to different concentrations, however, with the highest concentration of aminocarb, their population fell to 2.58% showing a significant reduction ($t=2.941$, $P<0.05$) compared to control. Regression between concentration of aminocarb and oenocytoid population yielded significant correlation coefficient (positive linear correlation, ($Y = 0.386 + 1815.0 X$, $r = 0.9527$, $P<0.001$). Similarly, the population of damaged/unidentified haemocytes also increased with the increasing concentration strength ($Y = 0.9395 + 4594.53 X$, $r = 0.9248$, $P<0.001$).

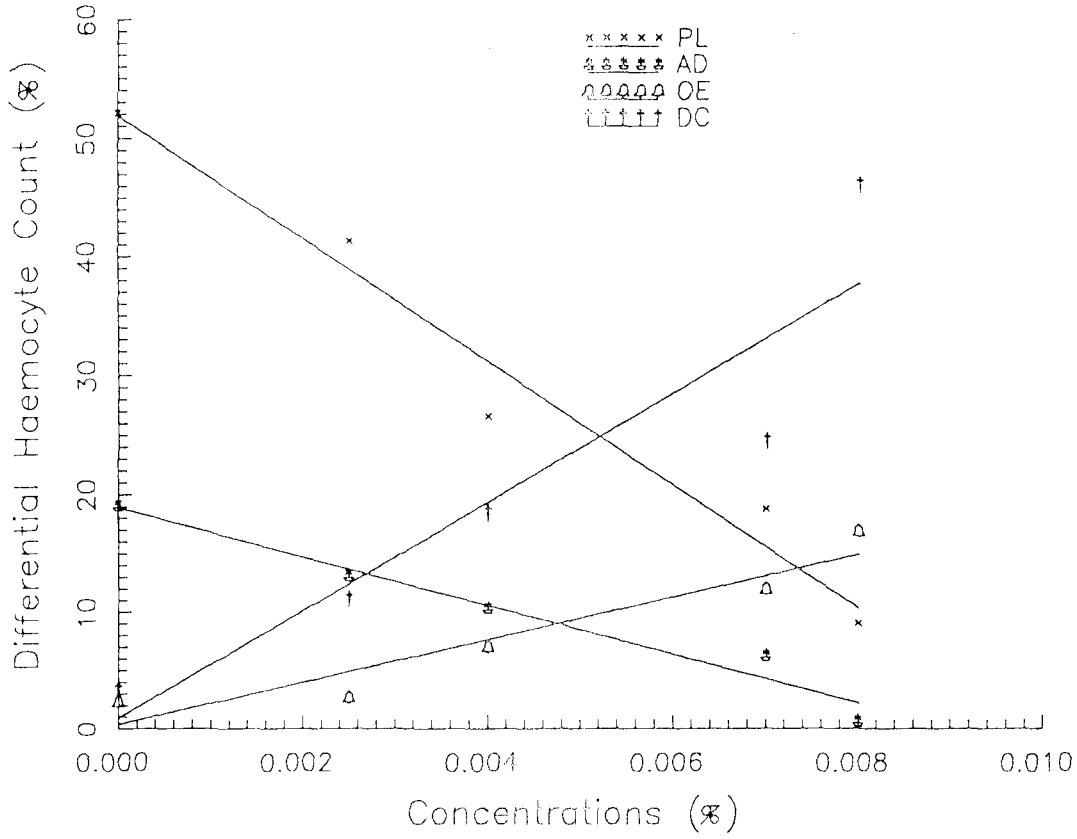

Fig. 5 : Correlation between the Differential Haemocyte Count (%) and various concentrations of aminocarb after 6 hrs of the treatment on one day old 5th instar nymphs of Dysdercus cingulatus.

After 1 day (Table-13, Fig.6)

After one day following treatment with different concentrations viz 0.0025, 0.004, 0.007 and 0.008% aminocarb, the prohaemocyte population was inconsistently increased, and respectively constituted 19.08%, 30.8%, 22.64% and 14.22% of total haemocytes compared to control (12.74%). On the other hand, plasmatocytes were 23.46%, 16.98% and 11.34% in the smears affected with 0.0025, 0.004 and 0.007% aminocarb undergoing statistically significant ($t=2.977$, $t=3.862$ and 3.929 respectively, $P<0.05$) reduction and therefore exhibiting negative linear correlation ($Y = 44.662 - 4310.33 X$, $r = - 0.9621$, $P<0.001$) with increasing concentration. Similar trend of reduction was observed in case of adipohaemocytes. Granulocytes were enhanced in number by the lowest concentration (0.0025%), however, the next two higher concentrations did not affect their population. Moreover, these haemocytes vanished from smears of nymphs affected with the highest concentration. Oenocytoids percentage was positively linearly correlated with the increasing concentration of aminocarb ($Y = -0.244 + 2271.59 X$, $r = 0.8915$, $P<0.001$). Similarly, following the application of 0.004, 0.007 and 0.008% aminocarb, the population of damaged cells increased to 17.94% 35.86% and 51.28%, respectively, showing a statistically significant enhancement ($t=3.470$, $t=4.405$ and $t=5.819$ respectively, $P<0.05$) compared to control (3.80%). Regression between percentage of these cells and concentration of aminocarb yielded positive linear correlation ($Y = - 1.244 + 5791.59 X$, $r = 0.9650$, $P<0.001$).

After 3 days (Table-14, Fig.7)

Three days following aminocarb treatment, prohaemocytes were inconsistently increased with respect to concentration strength. However, plasmatocytes displayed consistent but insignificant reduction due to great variation in the counts. Adipohaemocytes were completely damaged by two higher concentrations but granulocytes with the highest concentration (0.008%). Oenocytoids were increased from 3.44% in control to 11.52% in the smears of nymphs affected by the highest concentration showing positive linear correlation. ($Y= 3.018 + 1014.47 X$, $r = 0.9418$, $P<0.001$). Similar correlation was obtained in the proportion of damaged cells ($Y = -$

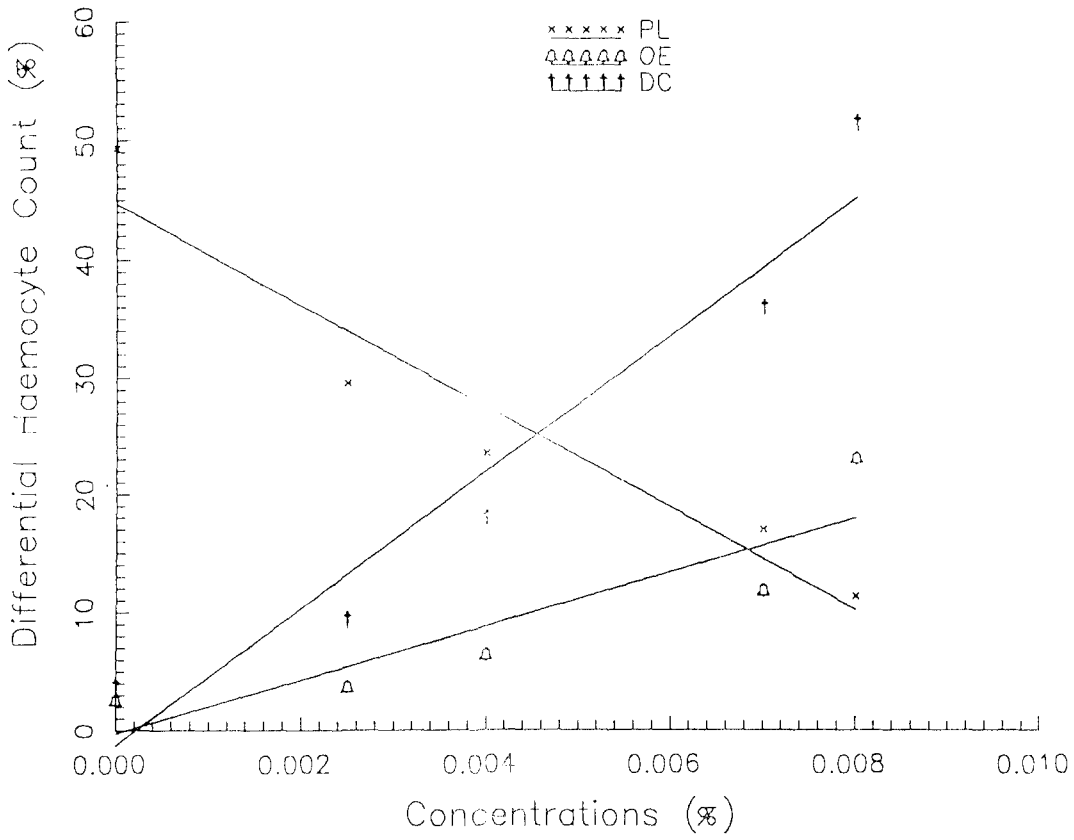

Fig. 6 : Correlation between the Differential Haemocyte Count (%) and various concentrations of aminocarb after one day of the treatment on one day old 5th instar nymphs of Dysdercus cingulatus.

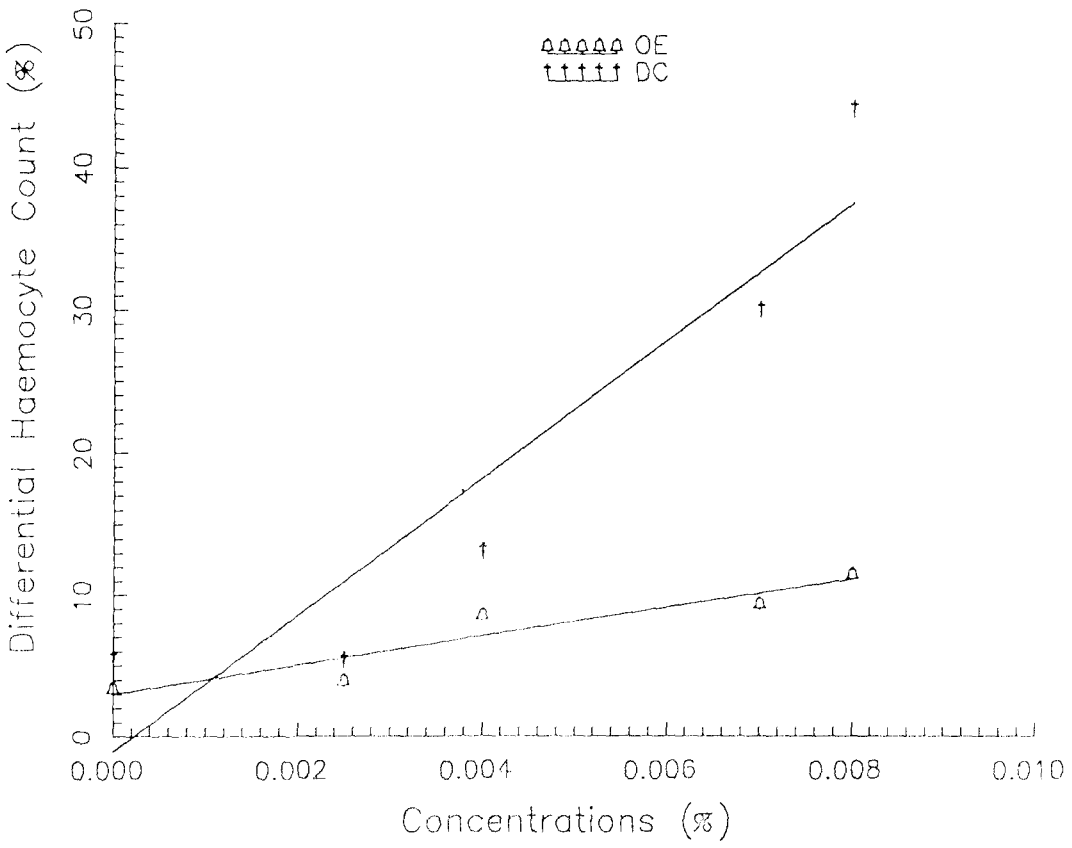

Fig. 7 : Correlation between the Differential Haemocyte Count (%) and various concentrations of aminocarb after 3 days of the treatment on one day old 5th instar nymphs of Dysdercus cingulatus.

1.069 + 4810.42 X, r = 0.9321, P<0.001). The two higher concentrations (0.007 and 0.008%) produced significantly high population of damaged cells (30.0%, t=3.645 and 43.94%, t=3.758, P<0.05), respectively.

After 5 days (Table-15, Fig.8)

After five days of treatment with increasing concentrations , the overall pattern of relative abundance of different haemocytes was found to be somewhat similar as mentioned earlier, therefore, displaying increase in prohaemocyte population, linear reduction in plasmatocytes (Y = 39.10 - 4024.63 X, r = - 0.9912, P<0.001), adipohaemocytes and granulocytes and apparent increase in oenocytoid percentage. In addition to that a significant linear increase (Y = 0.90 + 4948.60 X, r = 0.9657, P<0.001) in the percentage of damaged and disintegrating cells was observed with increasing concentration (10.14%, 15.24% 35.72% and 44.07%, respectively).

After imaginal ecdysis

Prohaemocyte and oenocytoid population was significantly high in the adult males emerged from nymphs treated with the highest concentration (0.008%) as compared to control (Table-16). All other haemocytes showed statistically insignificant change in their population.

Likewise in the females (Table-17) affected with aminocarb the prohaemocytes exhibited positive linear correlation with increasing concentration. Moreover, the 0.004 and 0 007% concentrations induced significant increase in their population (t=2.861 and t=3.544, P<0.05). Besides that, treatment with the 0.007% aminocarb caused significant reduction in plasmatocyte population. Densities of other haemocytes showed insignificant change in response to insecticidal treatment.

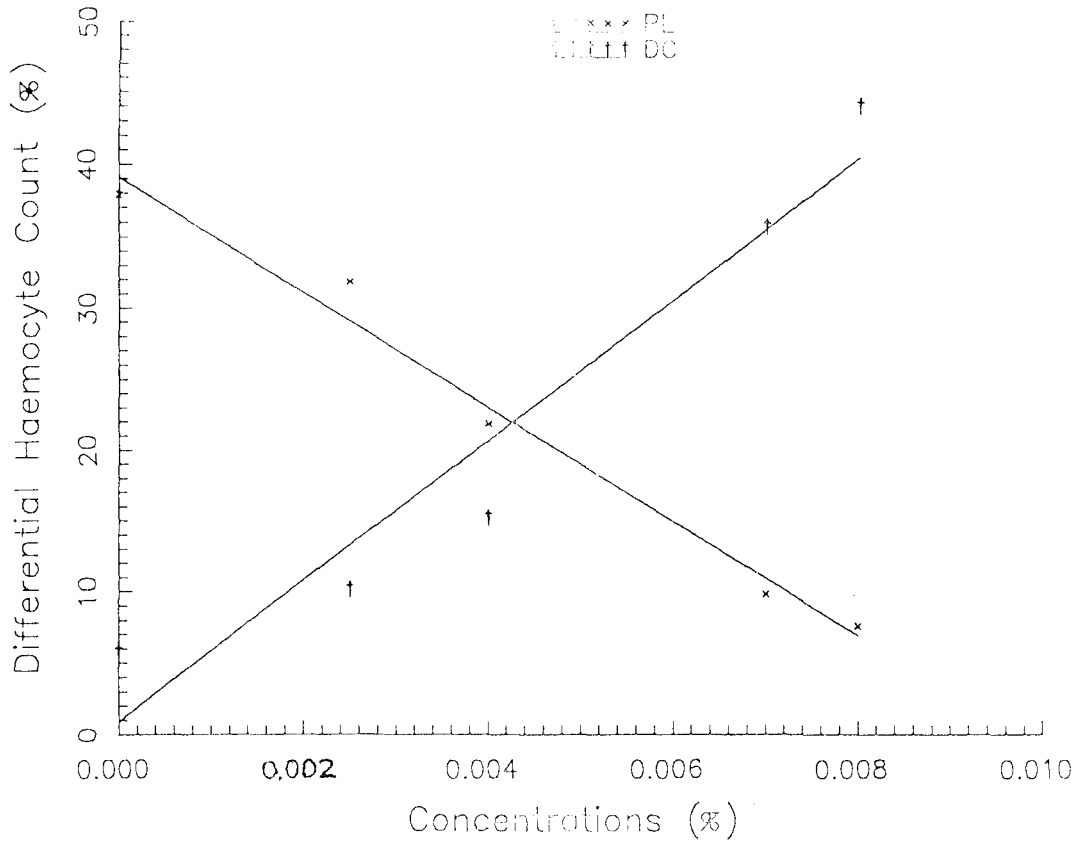

Fig. 8 : Correlation between the differential Haemocyte Count (%) and various concentrations of aminocarb after 5 days of the treatment on one day old 5th instar nymphs of Dysdercus cingulatus.

3. Effect of topical application of different concentrations of Cypermethrin (a pyrethroid) on *Dysdercus cingulatus.*

As mentioned in the cases of acephate and aminocarb, four concentrations of cypermethrin, from lower to higher, (0.0004, 0.0006, 0.0008 and 0.001%) caused approximately 25-30%, 45-50%, 65-70% and 85-90% mortalities in 24 hrs. In addition to that, about 1-2% treated insects died daily up to the emergence of adults. Treatment with 0.0004% cypermethrin resulted in very mild pharmacological symptoms, showing an early recovery from toxic effects. Next two doses induced moderate symptoms in the treated nymphs and the survived insects showed recovery in 2-3 days. Similarly, like acephate and aminocarb, cypermethrin at the highest selected concentration (0.001%) brought about severe pharmacological symptoms within 6 hrs of treatment as characterized by copious vomiting, anal discharge, inhibited feeding, twitching of legs and subsequent paralysis. Three days after treatment with the highest concentration extremely feeble, obviously dehydrated larvae displaying feeble tremors were found to contain very little haemolymph due to excessive water loss and insects appeared shrunken.

After 5 days, the haemolymph became highly viscous, the yield was low and insects could not survive beyond 5 days.

3.1 Haemocyte picture of 5th instar nymphs and adults of *Dysdercus cingulatus* affected with different concentrations of Cypermethrin.

Haemocytes of nymphs treated with different concentrations (0.0004, 0.0006, 0.0008 and 0.001%) of cypermethrin were examined in stained films after 6 hrs, and then after 1 day, 3 days, and 5 days following treatment. When the treated nymphs moulted to adult stage, haemocytes were studied in 1 day old males and the females.

Relatively few abnormal haemocytes were observed in 0.0004 and 0.0006% cypermethrin treated nymphs after 6 hrs. Prohaemocytes were almost unaffected. Adipohaemocytes showed large vacuoles (Plate-VI, Fig. A). In the nymphs topically applied with 0.0008% cypermethrin, highly variable numbers of grossly abnormal haemocytes were found in many cases, which displayed bizarre surface irregularities. (poikilo haemocytes), cytoplasmic and nuclear vacuoles, cytoplasmic disintegration, enlarged pycnotic or ruptured nuclei and abnormally stained cells (Plate-VI, Fig. B & D). It is important to point out that by far the greatest number of abnormal cellular changes observed during these studies occurred in the plasmatocytes. Granulocytes showed vacuolization of cytoplasm and discharge of granules. Generally oenocytoids and prohaemocytes were least affected, whereas, adipohaemocytes developed vacuoles and discharge of fat droplets into surroundings. However these cells were altogether absent from smears of nymphs affected with two higher concentrations (0.0008 and 0.001%).

After one day the changes in haemocytes were of similar nature following treatment with different concentrations of cypermethrin as found after 6 hrs. Besides that, mitotic figures were numerous. Following the application of higher concentrations, (0.0008 and 0.001%) adipohaemocytes were very few in smears. Plasmatocytes were more or less spread, having a round, ovoid or irregular shape and showed much evidence of surface activity in the form of very thin, lamellar, ectoplasmic extensions, numerous vacuoles in cytoplasm and nuclei, achromophilia and loss of normal structure. Accompanying these changes and the subsequent degenerative changes, agglutination occurred involving plasmatocytes and granulocytes. However, oenocytoids were slightly deformed and contained a few vacuoles (Plate-VI, Fig. C). Prohaemocytes largely showed abnormalities in nuclei.

After three days of application with lower concentrations, some haemocytes showed regenerative changes which were also evident by appearance of fusiform plasmatocytes and a large number of apparently normal prohaemocytes (Plate-VI, Fig. E).

Furthermore, following the application of the highest concentration, the active degenerative changes progressed in general, the normal cytoplasmic structure tended either to become disrupted and to assume an abnormal appearance or to become less visible until it completely disappeared and sometimes both (Plate-VI, Fig. G). Usually as degeneration progressed many of the affected cells showed poor staining. Some degenerating cells underwent a sort of deformation in which broad bulgings, suggestive of pseudopodia occurred. In some cases only such broad cytoplasmic bulges occurred but sometimes cells formed a greater number of smaller bulges or pseudopodia like extensions which gave the peripheries of the cells a very irregular aspect, as though the cells were extending fingers of cytoplasm from their surfaces (Plate-VI, Fig. F). Advanced mitotic stages were also, sometimes, encountered in these cells. The cells frequently took on a more ragged aspect as degeneration proceeded, the cytoplasm appearing irregularly frayed and torn. Degenerating cells were usually grossly vacuolated to an abnormal degree and showed varying degrees of nuclear disintegration (Plate-VI, Fig. H).

Treatment with lower concentrations of cypermethrin (0.0004 and 0.0006%) did not further induce any new abnormalities after 5 days. Some plasmatocytes showed large extensions of cytoplasm, whereas, a large population of prohaemocytes appeared like those of normal smears (Plate-VI, Fig. J). However, 0.0008% cypermethrin affected smears still showed various cytoplasmic and nuclear abnormalities in plasmatocytes and granulocytes (Plate-VI, Fig. I). Adipohaemocytes were either absent in smear or highly damaged. Oenocytoids showed usual deformities. Furthermore, the highest concentration (0.001%) resulted in marked hematological changes showing gross cytoplasmic abnormalities in all types of haemocytes (Plate-VI, Fig. K, L, M & N), very severe in plasmatocytes and granulocytes and milder in prohaemocytes and oenocytoids. The nuclei of degenerating cells became distorted, ragged, amorphous and achromophilic in most of the affected cells. In addition to that, large clumps of agglutinated cells were present. Even though severe degeneration was observed in most of the haemocytes, some appeared like those of the normal.

When the treated nymphs moulted to adult stage, a large population of damaged haemocytes was found to have vanished from the haemolymph. However, some plasmatocytes and adipohaemocytes still suffered from pathological symptoms such as vacuolization of cytoplasm, irregular cell membrane, cytoplasmic extension and extrusion of cytoplasm.

3.2 THCs of 5th instar nymphs and adults of *Dysdercus cingulatus* affected with different concentrations of Cypermethrin.

The THCs of affected nymphs were recorded at 6 hrs, 1 day, 3 days and 5 days after the treatment with each of the selected concentrations of cypermethrin on 1 day old 5thinstar nymphs. When these nymphs moulted to adult stage, the total haemocyte count was recorded in one day old males and females. The age-wise mean THCs are summarized in Table-3.

After 6 hrs

In the normal and solvent treated nymphs of the respective age THC was recorded as 4729.5 ± 930.05 cells/mm^3 and 4603.5 ± 456.04 cells/mm3 respectively. By the application of lowest concentration (0.0004%) there was slight albeit insignificant (7.62%) increase in cell population. However, by 0.0006 and 0.0008% cypermethrin there was a reduction of 10.94% and 35.29% in THC as compared to control. Furthermore, by the highest concentration (0.001%) the THC was reduced by 58.26% which was also insignificant at 5% level(t=2.7413, P>0.05).

After 1 day

After about 24 hrs following treatment with 0.0004% cypermethrin, the percent increase in cell population was slightly less than that found after 6 hrs. However, by 0.0006% cypermethrin, the reduction in THC was 39.93% (t=2.3424, P>0.05) which further reduced to 53.21% (t=2.3839,P>0.05) and 61.99% (t=2.9772,

P<0.05) by 0.0008% and 0.001% concentrations respectively as compared to the control.

After 3 days

In the normal and control nymphs the THC was 9121.5 ± 1255.54/mm^3 and 9367.5 ± 1077.14%/mm^3 of haemolymph, respectively. After 3 days following the treatment with 0.0004 and 0.0006% cypermethrin, there was a decrease in THC by 13.08 and 23.86% which was statistically insignificant. Whereas, the higher concentrations caused a statistically significant decrease by 60.18 and 70.79% (t=2.8428 and t=3.0374, P<0.05).

After 5 days

In the untreated and control nymphs of the respective stage, the THC was found to be 6273 ± 616.86 and 6376.5 ± 738.83 cells/mm^3. Nymphs subjected to the treatment with 0.0004, 0.0006 and 0.0008% cypermethrin exhibited a reduction in THC by 7.63, 18.77 and 37.19% which was comparatively less than the corresponding value after one day and 3 days indicating the possibility of regeneration of blood cells. However, with the highest dose, the reduction in THC was 66.97% (t=3.0767, P<0.05) which was slightly less than the percent loss of haemocytes after 3 days following treatment. The nymphs treated with highest concentration were completely paralysed and yielded very little haemolymph. The blood was of thick consistency due to water loss apparently caused by the action of insecticide. Clumps of agglutinated haemocytes were observed at many places in the smear indicating the increased tendency of blood coagulability. The nymphs treated with the highest dose could not survive beyond 5 days.

After imaginal ecdysis

The average THC of one day old adult males from untreated and acetone treated stock (control) was 3361 ± 402.78 and 3478.5 ± 496.31 cells/mm^3 , respectively. Subsequent to the treatment with 0.0004, 0.0006 and 0.0008%

concentrations of cypermethrin, the respective reductions in THCs were 5.49%, 12.72% and 19.01% which were statistically insignificant at 5% level. Following the highest concentration (0.001%) all the treated nymphs suffered mortality before imaginal ecdysis.

The THCs of 1 day old females of the untreated and solvent treated nymphal stock was found to be 6399 ± 756.07 and 6210 ± 521.98 cells/mm^3. In the females emerged from nymphs affected with 0.0004, 0.0006 and 0.0008% cypermethrin, the reduction in THC was by 0.65, 13.19 and 20.01%, respectively, which was statistically insignificant compared to control.

3.3 DHCs of 5th instar nymphs and adults of *Dysdercus cingulatus* affected with different concentrations of Cypermethrin.

DHC was recorded in the nymphs of *D. cingulatus*, after 6 hrs, 1 day, 3 days, 5 days following the treatment with 0.0004, 0.0006. 0.0008 and 0.001% concentrations of cypermethrin. when the treated nymphs moulted to adult stage the relative percentage of all types of haemocytes was recorded in adult males and the females.

After 6 hrs (Table-18, Fig.9)

The population of prohaemocytes was 2.1, 17.86, 22.12 and 23.88% more in the nymphs affected with 0.0004, 0.0006., 0.0008 and 0.001% cypermethrin, respectively, thus exhibiting a positive linear correlation ($Y = 7.07 + 27355.41 X$, $r = 0.9295$, $P<0.001$) with respect to increasing concentrations of cypermethrin, whereas, plasmatocytes showed negative linear correlation ($Y = 51.12 - 37606.76 X$, $r = 0.9266$, $P<0.001$).

Although lower concentrations of cypermethrin caused an insignificant reduction in adipohaemocyte population, higher ones completely wiped out these haemocytes from the smear. Similar trend was observed in granulocytes. The damaged cells exhibited a positive linear correlation with increasing concentrations of

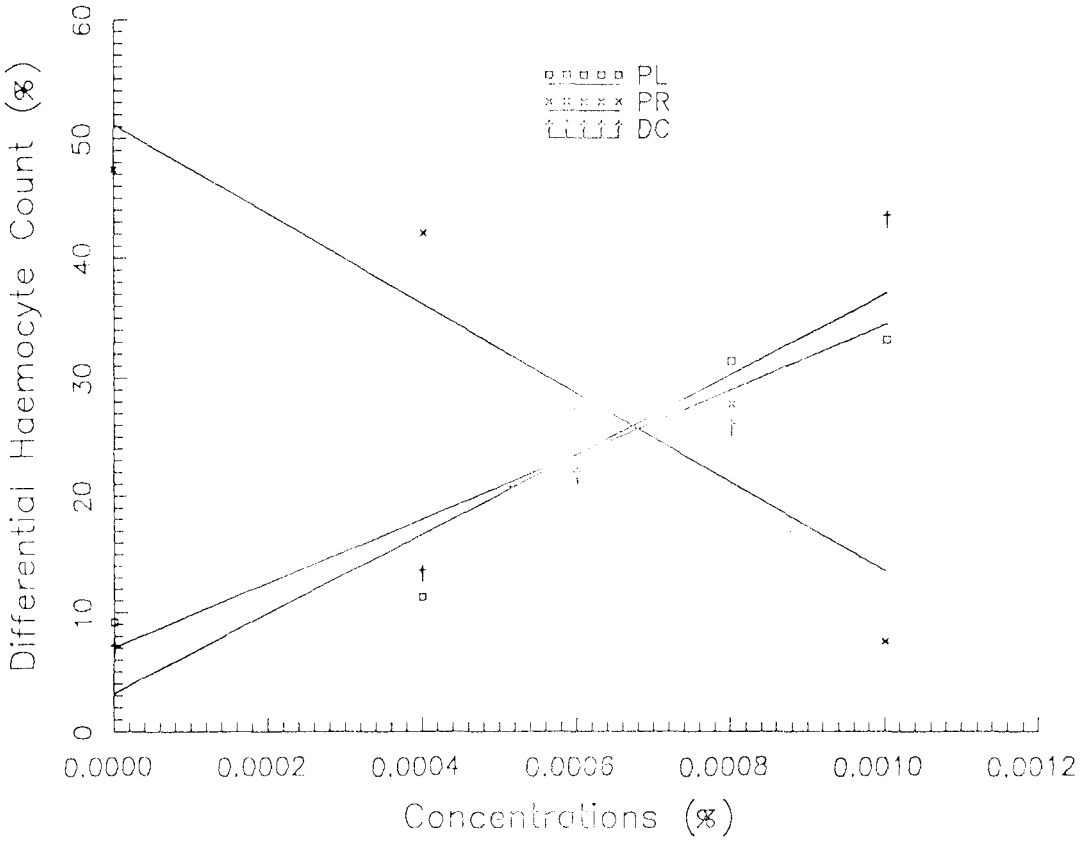

Fig. 9 : Correlation between the Differential Haemocyte Count (%) and various concentrations of cypermethrin after 6 hrs of the treatment on one day old 5th instar nymphs of Dysdercus cingulatus.

cypermethrin (Y= 3.21 + 33810.81 X, r = 0.9416, P<0.001). These cells were significantly high in the blood smears of 0.0006, 0.0008 and 0.001% cypermethrin (t=4.240, t=3.500 and t=4.234, P<0.05) treated nymphs.

After 1 day (Table-19, Fig.10)

Prohaemocytes showed inconsistent increase in their population with respect to increasing concentrations. On the other hand, plasmatocyte percentage was negatively linearly correlated with the increasing concentrations. With the application of 0.0008 and 0.001% cypermethrin, plasmatocytes were reduced to 11.32 and 4.66% (t=3.562 and t=4.143, P<0.05) showing a statistically significant reduction compared to control. Adipohaemocytes and granulocytes were completely damaged by the highest concentrations. Regression between concentration strength and number of damaged cells yielded positive linear correlation (Y= 1.176 + 44406.76 X, r = 0.9345, P<0.001).

After 3 days (Table-20, Fig.11)

Prohaemocytes exhibited insignificant increase in their population 3 days following cypermethrin treatment. Regression between concentration strength and plasmatocyte population yielded negative linear correlation(Y= 34.46 - 14183.78 X, r = - 0.9399, P<0.001). Adipohaemocytes were absent in the smears of 0.0008 and 0.001% cypermethrin treated nymphs. Granulocytes were slightly increased by 0.0004% cypermethrin, whereas, 0.0006 and 0.0008% concentrations caused reduction in their population. Furthermore, the highest concentration damaged these cells beyond recognition. Oenocytoid percentage was found to be increased with increasing concentration of cypermethrin. Regression between concentration strength and number of damaged cells yielded a positive significant linear correlation (Y= - 1.396 + 44835.14 X, r = 0.9434, P<0.001).

After 5 days (Table-21, Fig.12)

Five day after the treatment with different concentrations of cypermethrin, haemocytes followed the same trend as was found in cases of two previous

Fig. 7

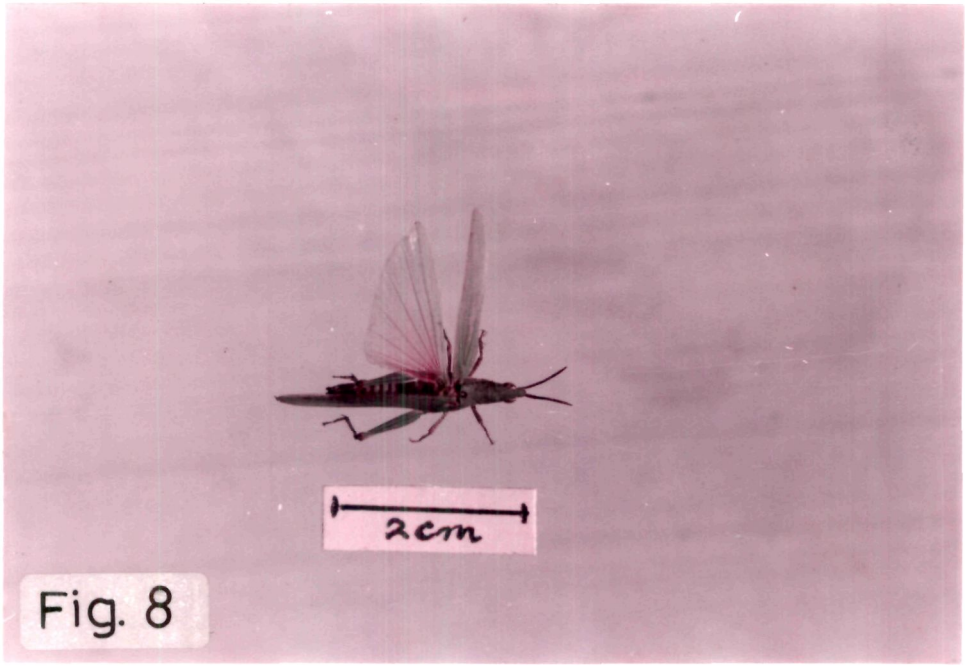

Fig. 8

Fig.9. *Tettix dorsifera* (Male)

Fig.10 *Tettix dorsifera* (Female)

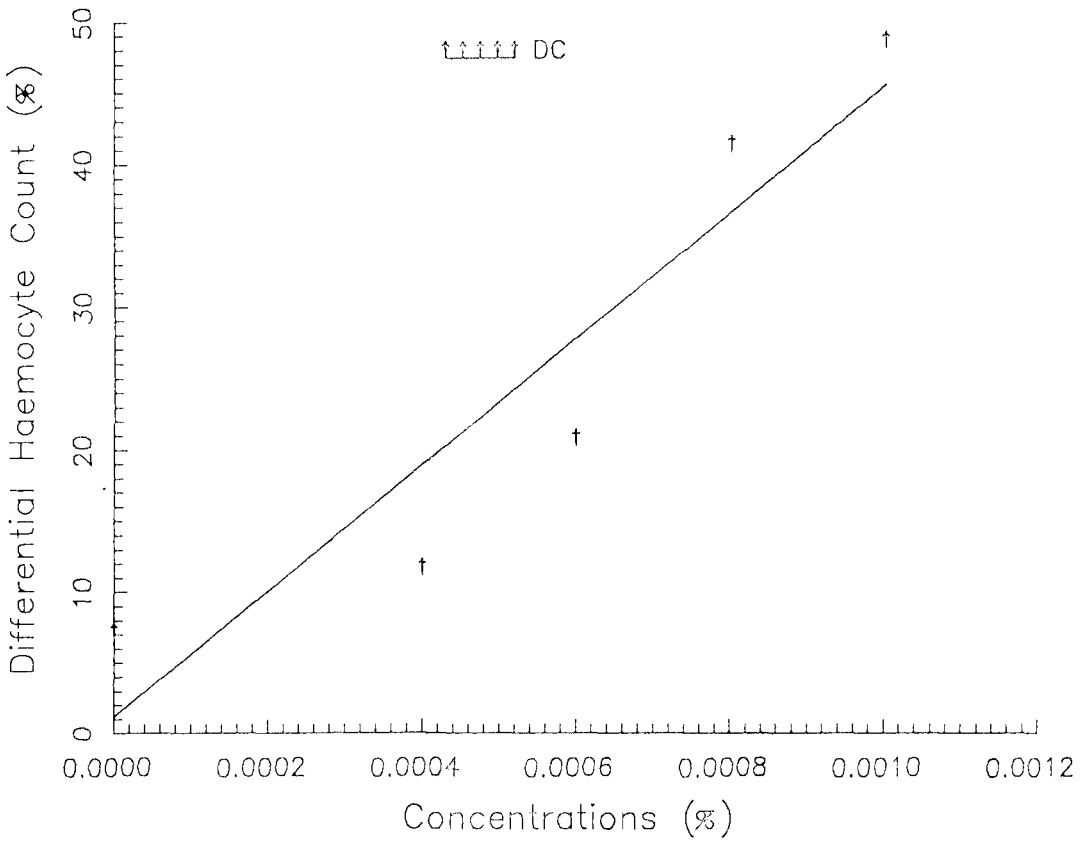

Fig. 10: Correlation between the Differential Haemocyte Count (%) and various concentrations of cypermethrin after one day of the treatment on one day old 5th instar nymphs of Dysdercus cingulatus.

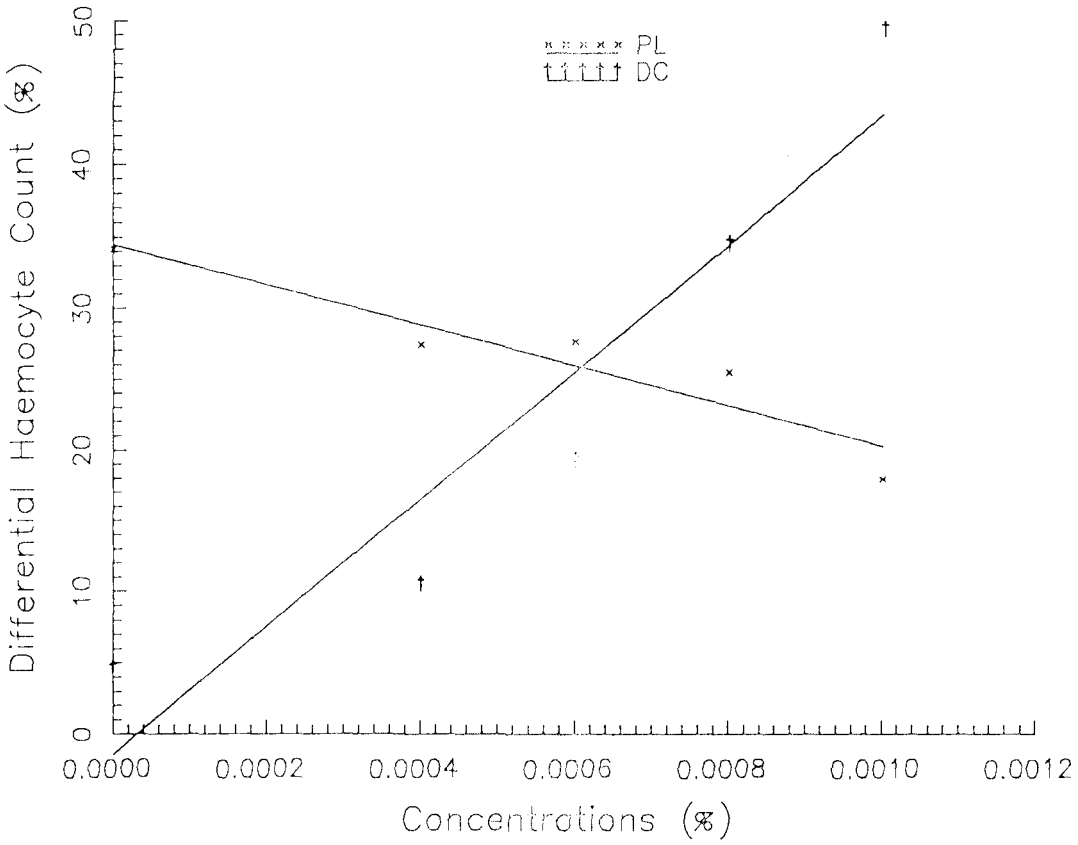

Fig. 11: Correlation between the Differential Haemocyte Count (%) and various concentrations of cypermethrin after 3 days of the treatment on one day old 5th instar nymphs of Dysdercus cingulatus.

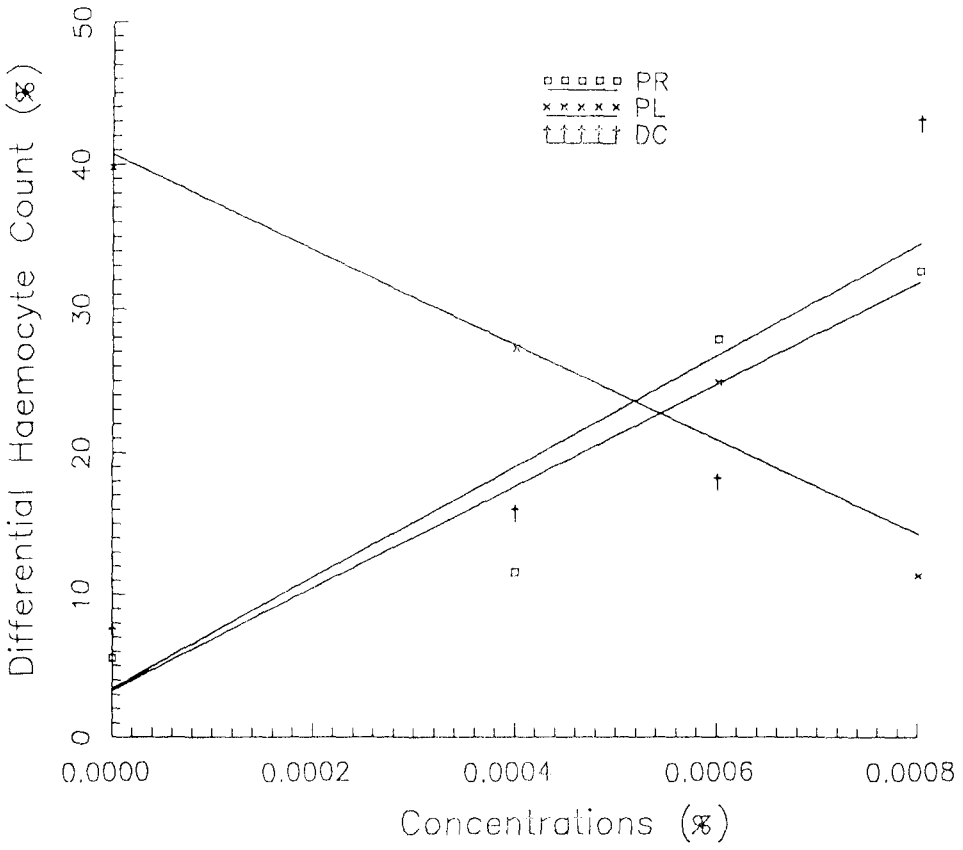

Fig. 12: Correlation between the Differential Haemocyte Count (%) and various concentrations of cypermethrin after 5 days of the treatment on one day old 5th instar nymphs of Dysdercus cingulatus.

insecticides. The prohaemocyte population yielded a positive linear correlation (Y = 3.25 + 36211.43 X, r = 0.9470, P<0.001), whereas, plasmatocyte showed negative linear correlation (Y= 40.72 - 32871.43 X, r = - 0.9669, P<0.001). The regression between damaged cell percentage and increasing concentration strength yielded a positive linear correlation (Y= 3.39 + 39331.43 X, r = 0.8692)

After imaginal ecdysis

Prohaemocytes, plasmatocytes and oenocytoids underwent insignificant alterations in their population in the adult males (Table-22) emerged from treated 5th instar nymphs. Adipohaemocytes were significantly low (17.34%, t=3.001, P<0.05) in 0.0008% affected one day old males compared to control (42.12%). On the other hand, granulocytes were significantly high (16.04%, t=3.020, P<0.05) in the smears of 0.0008% affected males. The population of damaged cells was significantly high (16.26%, t=3.335 P<0.05) at this concentration compared to control (5.58%).

The haemocytes of the affected females displayed insignificant changes in their differential counts (Table-23), however, number of damaged cells was significantly high (19.52%, t=3.0968, P<0.05) in 0.0008% affected females compared to control (6.40%).

4. Effect of topical application of different concentrations of Muristerone (a phytoecdysone) on *Dysdercus cingulatus.*

Four selected doses of muristerone were applied topically on individual 5th instar nymphs, 1-2 days old, and observations were made after 6 hrs, 1 day, 3 days and 5 days of treatment. The description of mortality, moulting and malformations after each time interval is given below.

Majority of nymphs treated with selected concentrations of muristerone apparently showed no visible signs of pharmacological symptoms after 6 hrs except displaying slow movements inside the jar.

The treated nymphs remained sluggish after 1 day following treatment with all selected concentrations. 5-10% mortality occurred by lower concentrations (0.2 and 0.4% respectively) and 15-20% by the 0.6 and 0.8% concentrations respectively. Feeding was slow.

After 3 days following treatment with 0.2 and 0.4% muristerone nymphs were still sluggish and mortality was observed in 5-10% more nymphs. Feeding was occasional. Severe pharmacological symptoms were not noticed even after 3 days of application. Approximately 15% of the treated nymphs suffered mortality at 0.6% concentration, whereas, the highest concentration (0.8%) resulted in 20% mortality.

After 5 days of treatment with muristerone, moulting started to occur in majority of the nymphs. Subsequently, 10-20% nymphs succumbed to abnormal moulting in which nymphs were not able to free themselves from old cuticle. Moreover, 10-20% affected nymphs moulted to nymphal-adult intermediates at 0.2 and 0.4% concentration. On the other hand with the application of higher concentrations 25-30% of the treated nymphs succumbed to incomplete moulting and the survived ones moulted to nymphal-adult intermediates. These adults possessed characters of nymphs as well as adults. Their size was similar to 5^{th} instar nymphs, the wings were small and folded as well as only slightly larger than the wing pads of 5^{th} instar nymphs. Feeding and other activities including movements of nymphal adult intermediates were normal. However, flying ability was completely impaired. In addition to that there were several anatomical anomalies. These had a small undifferentiated, irregular mass of tissue in place of male or female reproductive organs. This tissue was soft and creamy white and had no resemblance to normal reproductive organs.

Thus nymphs subjected to higher concentrations of muristerone (0.6 and 0.8%) did not transform to normal adults after moulting. Those subjected to lower concentrations (0.2 and 0.4%) moulted to 20-30% apparently normal adults with no morphological abnormalities.

4.1 Haemocyte picture of 5th instar nymphs and adults of *Dysdercus cingulatus* affected with different concentrations of Muristerone.

The observations made on the haemocyte histopathology in the nymphs and adults after 6hrs. 1 day, 3 days, 5 days following the treatment with various concentrations of muristerone are given below.

Haemocytes of nymphs affected with lower concentrations did not exhibit any appreciable alterations in their normal morphology after 6 hrs of treatment, whereas, higher concentrations of muristerone induced cytoplasmic vacuolization in a fair population of adipohaemocytes and plasmatocytes. Only a few prohaemocytes were highly affected and displayed surface irregularities with few vacuoles in cytoplasm (Plate-VII, Fig. A & B). Nuclei of these cells became partially or completely extruded. Occasionally, the extruded nuclei seemed to be in mitotic condition. oenocytoids appeared like those of normal.

One day following the treatment with lower concentrations, a large number of haemocytes appeared normal. Some adipohaemocytes and plasmatocytes showed cytoplasmic and nuclear vacuolization. Adipohaemocytes containing no visible nuclei were occasionally observed. Nucleic of degenerating cells sometimes appeared to breakup into a number of discrete bodies (Plate-VII, Fig. G & H). With the higher concentrations, the cytoplasmic and nuclear abnormalities spread to some more haemocytes. The plasmatocytes and prohaemocytes occasionally exhibited somewhat a swollen aspect which was also observed in the nuclei of some oenocytoids (Plate-VII, Fig. F). Severe degenerative changes in haemocytes, as found in the cases of acephate, aminocarb and cypermethrin, were, however, not generally observed and if present, were confined to only a few plasmatocytes and granulocytes.

Blood smears of nymphs affected with lower concentrations, after 3 days of treatment showed slight cytoplasmic and nuclear abnormalities in a few haemocytes characterized by rounding up of plasmatocytes some cell agglutination; evident by sporadic clumps of haemocytes in the smear, and some cell disintegration (Plate-VII, Fig. D). Furthermore, haemocytes of nymphs subjected to higher concentrations

displayed more cytoplasmic and nuclear abnormalities compared to control as well as lower concentrations particularly with respect to rounding up, agglutination and increased vacuolization. Increased mitotic activity was recorded in prohaemocytes and plasmatocytes (Plate-VII, Fig. C & E).

After five days following 0.2 and 0.4% muristerone, the adipohaemocytes showed large vacuoles in cytoplasm. The plasmatocytes were mostly round or oval, containing numerous small vacuoles in cytoplasm, appeared more or less spread (Plate-VII, Fig. I), and showing surface activity in the form of very thin, lamellar, ectoplasmic extensions, some of which were relatively large.

Haemocytes of adult males and females affected with 0.2 and 0.4% muristerone appeared to be unchanged. However, there was an apparent increase in mitotic activity, which was evident in the nuclei of some cells especially prohaemocytes and plasmatocytes by assuming an appearance, suggestive of the prophase of mitosis. Other advance phases of mitosis were also observed in blood smears.

Treatment of nymphs with higher concentrations of muristerone resulted in precocious moulting which subsequently caused formation of nymphal adult intermediates. The damage induced by 0.8% muristerone was mostly confined to plasmatocytes and granulocytes (Plate-VII, Fig. J & K). Unlike the blood smears of nymphs subjected to higher concentrations of acephate, aminocarb & cypermethrin, muristerone did not destroy the adipohaemocytes completely. Consequently, a large number of these cells were present in smears of intermediates. Oenocytoids and majority of prohaemocytes appeared to be unaffected even at the highest concentration of muristerone (Plate VII, Fig. L). Occasionally some disintegrating haemocytes were also observed (Plate-VII, Fig. M).

4.2 THCs of 5th instar nymphs and adults of *Dysdercus cingulatus* affected with different concentrations of Muristerone.

The THCs of affected nymphs were recorded after 6 hrs, 1 day, 3 days and 5 days following treatment with graded concentrations of muristerone. The THCs of 1 day old adult males and females and nymphal-adult intermediates were also recorded. The data is tabulated in table-04.

After 6 hrs

The total haemocyte count showed an insignificant increase of 3.99% and 9.55% following the treatment with 0.2 and 0.4% muristerone. With the higher concentrations the reduction in THCs was 33.49% and 40.73%, respectively, compared to control. In the control nymphs of respective age, the THC was 4851 ± 788.13 cells/mm^3 ,whereas, in untreated insects the THC was slightly less showing 4549.5 ± 378.67 cells/mm^3 of blood.

After 1 day

After 24 hrs following treatment with 0.2% muristerone. THC exhibited an increase of 12.47% which was statistically insignificant. Besides, following the treatment with 0.4, 0.6 and 0.8% muristerone, the reduction in THC was by 15.86% (t=1.8782, P>0.05), 39.64% (t=2.5726, P>0.05) 52.43% (t=3.0689, P<0.05) respectively as compared to control. THCs of untreated and solvent treated nymphs (control) of the corresponding age, respectively, were 6988.5 ± 773.57 and 7038 ± 346.61 cells/mm^3.

After 3 days

The THCs of untreated and solvent treated nymphs (control) of respective age and stage were 9531 ± 653.91 and 9688.5 ± 440.02 cells/mm^3. After 3 days following the treatment with 0.2 and 0.4% muristerone. THC was respectively enhanced by

14.82 and 31.58% compared to control but this increase was statistically insignificant at 5%level. The total counts of individual insects varied highly. Moreover, two higher concentrations i.e. 0.6 and 0.8% induced a reduction of 31.73% ($t=2.5511$, $P>0.05$) and 57.73 ($t=3.6435$, $P<0.05$) in THC as compared to control.

After 5 days

The THCs of untreated and solvent treated nymphs (control) of corresponding age were found to be 6075 ± 768.73 and 6228 ± 362.76 per mm^3. The treatment with two lower concentrations i.e. 0.2 and 0.4% caused statistically insignificant reduction in THC by 9.83 and 20.73% respectively. However, the treatment with 0.6 and 0.8% concentration resulted in acceleration in moulting, consequently, majority of the treated nymphs moulted to intermediates (nymphal-adult) after 3-5 days following the application. The average THCs of these intermediates were 5940 ± 478.52 and 3955.5 ± 593.91 mm^3 of haemolymph respectively by 0.6 and 0.8% muristerone.

After imaginal ecdysis

One day old adults emerged from untreated and acetone treated nymphs respectively showed 3663 ± 611.17 and 3649.5 ± 593.39 cells/mm^3 of haemolymph. The adult males showed enhanced THC (16.77% and 24.29% respectively) by 0.2 and 0.4% concentrations of muristerone. Adult emergence did not occur in 0.6 and 0.8% muristerone treated nymphs. The females of untreated and acetone treated stock respectively had 6300 ± 505.22 cells/mm^3 and 6574.5 ± 597.81 cells/mm^3 .The treatment with 0.2 and 0.4% muristerone enhanced the cell count by 5.20 and 11.09% in comparison to control.

4.3 DHCs of 5th instar nymphs and adults of *Dysdercus cingulatus* affected with different concentrations of Muristerone

Following application of four selected concentrations of muristerone (0.2, 0.4, 0.6 and 0.8%) on 5th instar nymphs the differential counts were recorded after 6 hrs., 1 day, 3 days and 5 days of treatment. Following ecdysis, DHCs were determined in one day old males and females and intermediates (nymphal- adults).

After 6 hrs (Table-24, Fig.13)

Prohaemocytes were reduced to 6.36, 5.66 and 4.66% with the respective treatments with 0.4, 0.6 and 0.8% muristerone compared to 9.66% in control. Similarly, plasmatocytes exhibited changes in narrow range and were statistically insignificant. Muristerone resulted in enhancement of adipohaemocytes from 14.5% in acetone treated nymphs to 19.84, 21.56 and 23.24% in the affected nymphs with 0.2, 0.4 and 0.6% concentrations. However, with the highest concentration their population was reduced to half of the control value. The relative population of granulocytes was also reduced by all selected concentrations, however, showing inconsistent reduction with respect to increasing concentrations. Oenocytoids did not show much variation by lower concentrations but with higher doses their population became almost double. Regression between concentration strength and damaged cells population yielded a positive linear correlation ($y = 4.66 + 22.10 X$, $r = 0.9834$, $P<0.001$). The 0.6 and 0.8% muristerone induced a statistically significant increase in the number of damaged haemocytes (20.02%, $t=3.1790$ and 21.06%, $t= 3.3418$, $P<0.05$).

After 1 day (Table-25, Fig.14)

After one day following treatment, prohaemocytes showed slight reduction in their population. Plasmatocytes, on the other hand, exhibited slight increase in their population. Adipohaemocyte population was reduced to 9.08% from 20.78% in control showing statistically significant reduction followed by treatment with the highest concentration (0.8%) of muristerone. Granulocytes expressed a mixed response to

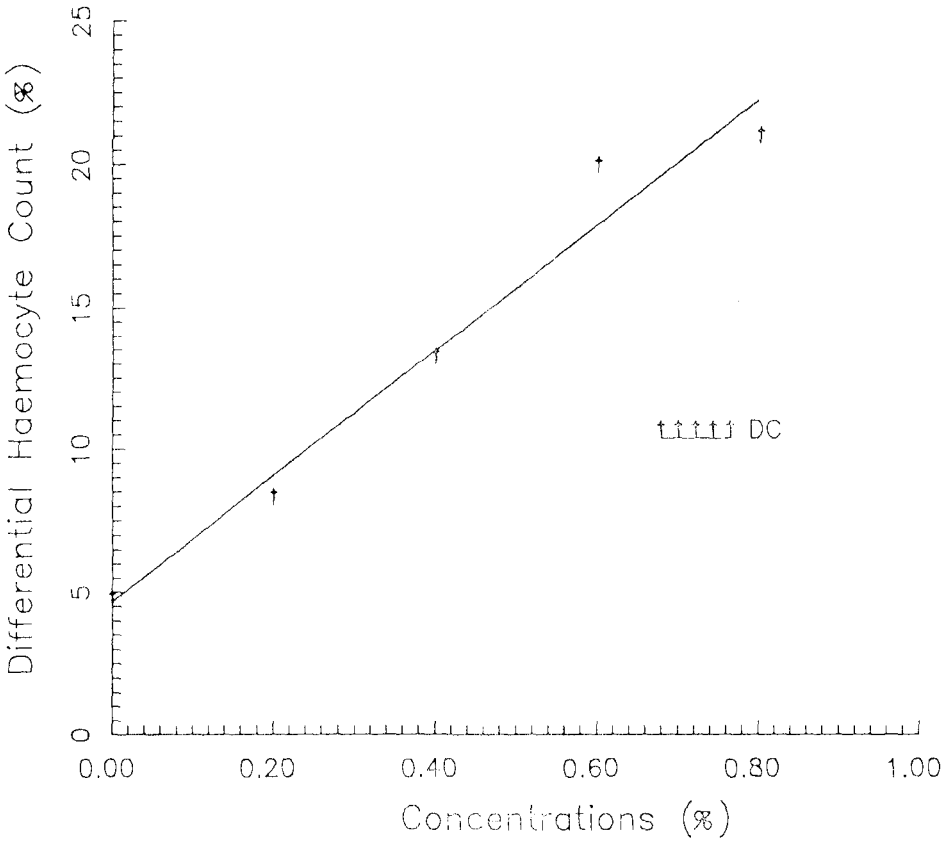

Fig. 13: Correlation between the Differential Haemocyte Count (%) and various concentrations of muristerone after 6 hrs of the treatment on one day old 5th instar nymphs of Dysdercus cingulatus.

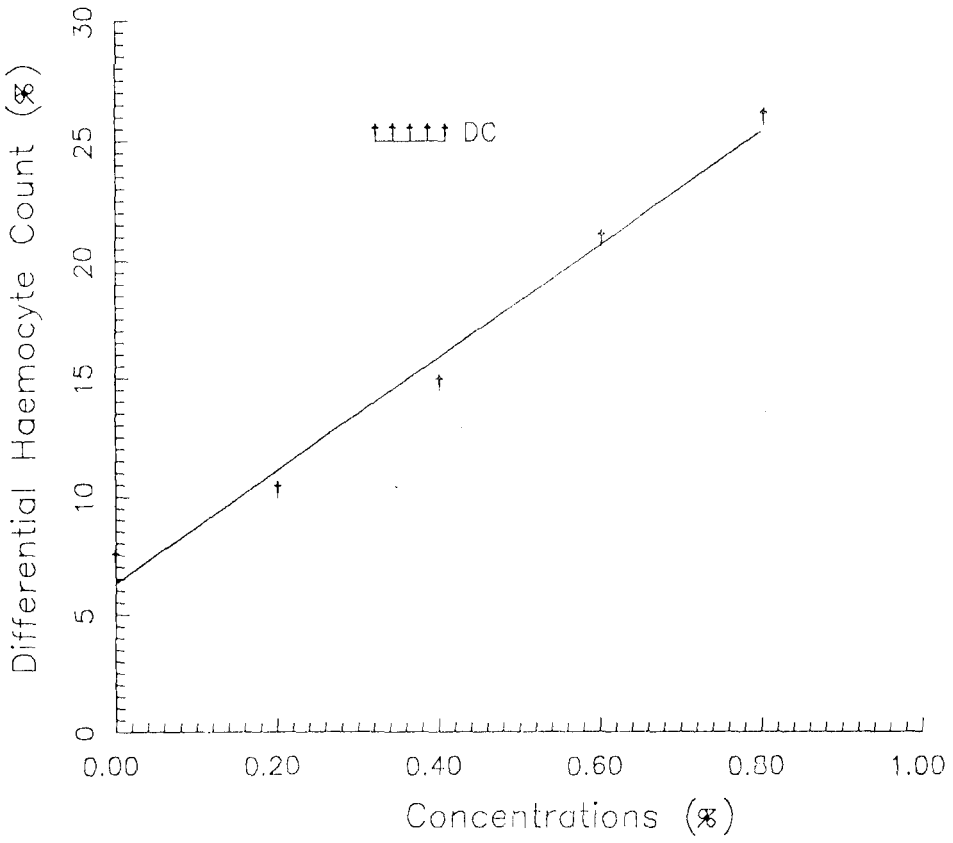

Fig. 14: Correlation between the Differential Haemocyte Count (%) and various concentrations of muristerone after one day of the treatment on one day old 5th instar nymphs of Dysdercus cingulatus.

muristerone treatment after one day. Oenocytoids exhibited only slight variation in their population showing either a little increase or reduction in their percentage which was insignificant statistically. The density of damaged cells showed a concentration based positive correlation ($Y= 6.312 + 23.93 X$, $r = 0.9927$, $P<0.001$) and increased to three times of the control, when treated with the 0.8% muristerone.

After 3 days (Table-26, Fig.15)

Prohaemocyte population did not show any remarkable change with the application of any of the selected concentration in comparison to control. Plasmatocytes exhibited insignificant increase in their relative population. Adipohaemocytes percentage indicated a negative linear correlation ($Y= 32.24 - 22.90 X$, $r = - 0.9521$, $P<0.001$) with increasing concentrations as compared to control and the highest concentrations significantly reduced their population from 30.60% (control) to 11.06% ($t=3.5437$, $P<0.05$). Granulocyte showed slight increase at the lowest concentration, however, progressively increasing concentrations induced reduction in their population. The two higher concentrations 0.6 and 0.8% caused a statistically significant reduction (2.84% $t= 3.1025$, $P<0.05$ and 1.82%, $t= 3.1684$, $P<0.05$ respectively). Oenocytoids showed only negligible changes compared to control. The percentage of damaged cells showed concentration based linear increase ($Y= 07.13 + 24.36 X$, $r = 0.9838$, $P<0.001$) reaching as high as 26.52% with the highest concentration of muristerone compared to 8.60% in control thus undergoing a statistically significant enhancement ($t= 3.1474$, $P<0.05$).

After 5 days (Table-27)

The nymphs treated with the higher concentrations started moulting to next stage after 5 days of treatment.

After imaginal ecdysis

Prohaemocytes were significantly more in the adult males which emerged from nymphs treated with 0.2 and 0.4% muristerone (12.24% $t=3.3234$, $P<0.05$ and

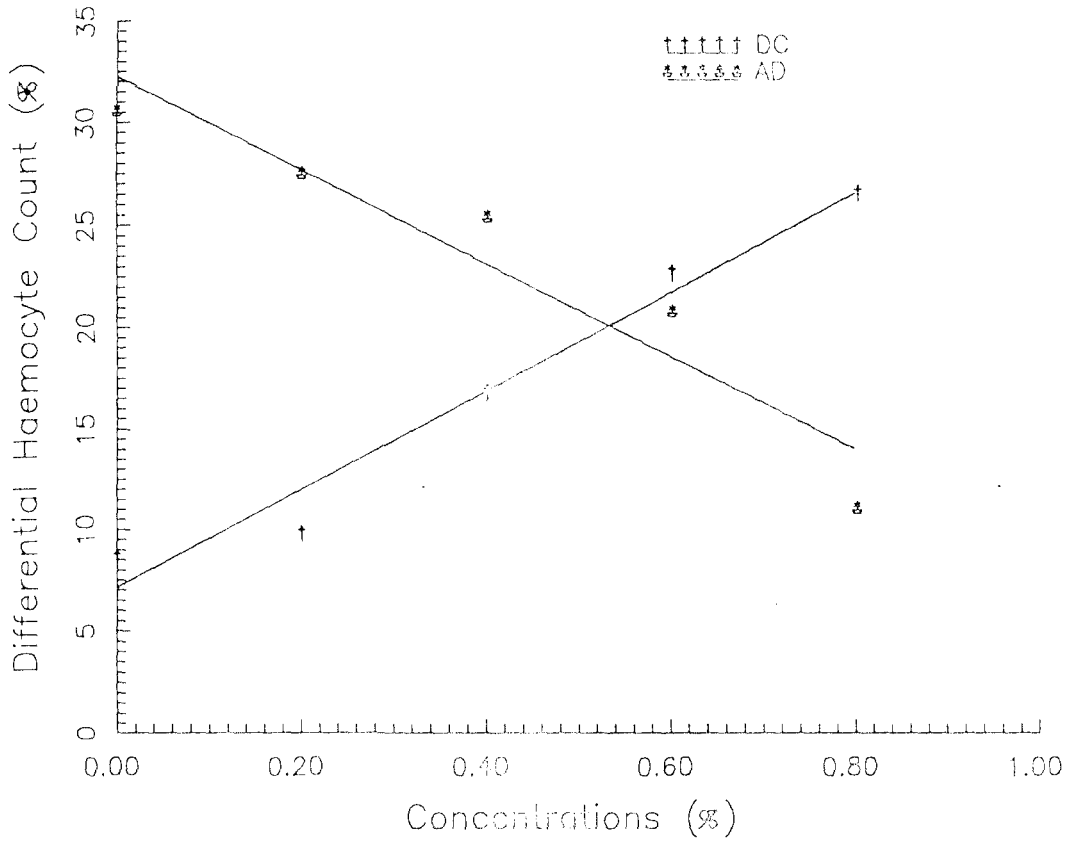

Fig. 15: Correlation between the Differential Haemocyte Count (%) and various concentrations of muristerone after 3 days of the treatment on one day old 5th instar nymphs of Dysdercus cingulatus.

16.38%, t= 3.7772, P<0.05) compared to control (2.94%). Even though insignificant statistically, plasmatocytes exhibited enhancement in their percent population. Since proportion of prohaemocytes and plasmatocytes underwent an increase, adipohaemocytes became relatively low at the above mentioned concentrations of muristerone. On the other hand, granulocytes and damaged cells showed enhancement albeit insignificant at 5%level (Table-28).

The smears of affected females (Table-29) emerged from nymphs treated with 0.2 and 0.4% muristerone consisted of 14.20 and 17.28% prohaemocytes compared to control (4.66%) showing statistically significant increase (t=2.8727, and t=3.5560, P<0.05 respectively). Plasmatocytes showed negligible variation in their relative proportion. Although adipohaemocytes and granulocytes were progressively less in population following increasing concentrations of muristerone, the reduction was statistically insignificant. The damaged/ unidentified cell were proportionately more compared to control.

Treatment with higher concentrations (0.6 and 0.8%) of muristerone to 5th instar nymphs resulted in the formation of intermediates (nymphal-adult) after the next moulting. Blood smear examination of these intermediates displayed respectively 5.26 and 5.28% prohaemocytes, 50.10 and 55.68% plasmatocytes, 14.94 and 7.40% adipohaemocytes, 4.30 and 4.42% granulocytes, 5.02 and 2.14% oenocytoids and 13.18 and 18.16% damaged cells.

5. Effect of topical application of different concentrations of Methoprene (a juvenoid) on *Dysdercus cingulatus*.

Like muristerone, methoprene (an analogue of juvenile hormone) too, belongs to the group of chemicals known as insect growth regulators . The treatment with various selected concentrations of methoprene viz 0.2, 0.1, 0.08 and 0.04% on 1-2 days old 5th instar nymphs respectively caused 20, 12, 6 and 4% mortalities after 1 day .

After 3 days of application of different concentrations total mortality increased to 31, 22, 15 and 12% After 5 days some more nymphs succumbed to hormone treatment thereby enhancing the total loss of lives to 39, 32, 20 and 17% respectively with 0.2, 0.1, 0.08 and 0.04% methoprene per nymph. In addition to the above mentioned mortalities approximately 25, 19, 12 and 9% of the total treated nymphs succumbed to abnormal moulting. Moreover, the nymphs subjected to the higher concentrations of methoprene (0.2 and 0.1%) transformed to nymphal-adult intermediates. Since all the survived and subsequently ecdysed nymphs transformed to intermediates by two higher concentrations, normal adult males and females did not emerge. These nymphal-adult intermediates possessed characteristics of nymphs as well as adults. They were larger in size than 5th instar nymphs, and their abdomen was of almost same size as that of adults but the wings were very small and folded and were slightly larger than the wing pads of 5th instar nymphs. Feeding and other activities of these intermediates were normal, however, flying ability was completely impaired with respect to the lower concentrations of methoprene. The successfully moulted nymphs transformed to adult males and females with or with out moderate wing abnormalities as the case may be. Approximately 15-25% adults, depending on dose applied, had wing abnormalities of varying intensities. Moreover, compared to normal adults, the intermediates had a small undifferentiated irregular mass of tissue in place of the female or the male reproductive organs. This tissue was soft and creamy white and had no resemblance to the normal reproductive organs.

5.1 Haemocyte picture of 5th instar nymphs and adults of *Dysdercus cingulatus* affected with different concentrations of Methoprene.

Following the topical application of different selected concentrations of methoprene viz., 0.04, 0.08, 0.1 and 0.2% on 5th instar nymphs of *Dysdercus cingulatus*, the permanent, stained blood smears were subsequently examined after 6 hrs, 1 day, 3 days and 5 days. Furthermore, the blood smears of adult males and

females as well as those of nymphal-adult intermediates were also examined to get the haemocyte profiles.

Haemolymph of nymphs affected with lower concentrations after 6 hrs contained haemocytes with no appreciable morphological abnormalities but the smears, affected with higher concentration viz. 0.1 and 0.2% methoprene were found to contain a large population of adipohaemocytes with big vacuoles and granulocytes with moderate cytoplasmic and nuclear abnormalities. Some plasmatocytes suffered slight vacuolization, majority of them were like those of normal nymphs (Plate-VIII, Fig. D & E).

Blood smears obtained 1 day after the treatment contained a large population of vacuolated adipohaemocytes and granulocytes. Some plasmatocytes, too, displayed excessive vacuolization in cytoplasm, and nucleus (Plate-VIII, Fig. G & I). However, majority of plasmatocytes appeared like those of normal nymphs. oenocytoids were entirely unaffected (Plate-VIII, Fig. K). Prohaemocytes too were quite normal, however, majority of them were very small in size and appeared as if were freshly released into haemolymph. Different stages of mitotic cell division were observed in the affected haemocytes (Plate-VIII, Fig. B).

After 3 days following treatment no new abnormalities appeared in haemocytes. The blood picture of affected nymphs with various concentrations was almost similar to that obtained after one day following the treatment. The highest concentration (0.2%) induced vacuolization in cytoplasm and nucleus of granulocytes (Plate-VIII, Fig. F) as well as nuclear deformities whereas oenocytoids were only slightly affected (Plate-VIII, Fig. J).

After five days following the treatment with higher concentrations, the smears of affected nymphs contained many immature prohaemocytes which appeared to be newly differentiated and freshly released into blood. Oenocytoids did not show any histopathological changes. The adipohaemocytes exhibited large vacuoles in cytoplasm. Moreover some of these haemocytes were so damaged that only naked nuclei were visible in the smear. Plasmatocytes displayed mild surface abnormalities

(Plate-VIII, Fig. A & C). Granulocytes affected with higher concentrations of methoprene exhibited marked cytoplasmic and nuclear deformities (Plate-VIII, Fig. H). The damaged cells displayed gross nuclear as well as cytoplasmic abnormalities (Plate-VIII, Fig. C).

The adult males and the females, from which haemolymph was obtained to make blood smears, also included those adults which showed marked wing abnormalities. Adult emergence took place in the nymphs treated with lower concentrations (0.04 and 0.08%) only. Slight hematological abnormalities, characterized by vacuolization in certain adipohaemocytes (Plate-VIII, Fig. M), as well as increased granulation, spreading and rounding up of plasmatocytes were noticed in blood smears of both males and females. Mitosis was frequently observed in prohaemocytes and plasmatocytes. Haemolymph smears of nymphal-adult intermediates contained a large population of plasmatocytes and adipohaemocytes. Some of these cells were with moderate cellular abnormalities (Plate-VIII, Fig. N). Mitosis was observed in 3-4% cells.

5.2 THCs of 5th instar nymphs and adults of *Dysdercus cingulatus* affected with different concentrations of Methoprene.

The THCs of affected nymphs were recorded after 6 hrs, 1 day, 3 days and 5 days following treatment with the four respective selected concentrations of methoprene. After imaginal moulting of the treated nymphs, the population of total cells was determined in one day old adult males and the females. The counts of affected insects were compared with those of control and are summarized in table-5.

After 6 hrs

The THCs of untreated and solvent treated nymphs (control) control of the corresponding age were 4405.5 ± 285.53 and 4800 ± 431.59 cells/mm^3. Following the treatment with 0.04 and 0.08% methoprene, the THC was increased by 1.81 and 6.87% of the control. Furthermore, by 0.1% methoprene there was slight reduction in

THC, however, treatment with the highest dose further reduced the THC by 31.38% in comparison to control.

After 1 day

One day after the treatment with 0.04, 0.08, 0.1% concentrations of muristerone, the variation in THC was within normal limits. However, by 0.2% concentration, THC of affected nymphs was 5049 ± 320.31 cells which was 18.99% less than the control.

After 3 days

After 3 days following the application of 0.04, 0.08 and 0.1% methoprene on 5^{th} instar nymphs of *D. cingulatus*, THC was reduced by 3.32, 13.93 and 26.34% respectively as compared to control. In the acetone treated nymphs (control) and untreated nymphs the respective THCs/mm^3 of blood were 9360 ± 436.06 and 9500 ± 455.76 . The application of highest concentration (0.2%) of methoprene resulted in the reduction of total count of haemocytes by 36.67% ($t=3.4288$, $P<0.05$).

After 5 days

THCs of untreated and acetone treated (control) 5^{th} instar nymphs of respective age were found to be 5958 ± 400.94 and 6300 ± 214.05 cells/mm^3 respectively. When the treatment was made with 0.04, 0.08 and 0.1% methoprene THCs were subsequently reduced by 1.57, 12.57 and 17.14%, respectively, after 5 days of application. The highest concentration (0.2%) of methoprene resulted in a reduction of 24.86% ($t=2.8090$, $P<0.05$) in total haemocytes per mm^3 of haemolymph in comparison to control.

After imaginal moulting

The adult males emerged from untreated and acetone treated nymphs had an average of 3560.5 ± 384.05 and 3775 ± 208.58 cells/mm3 respectively whereas

following the treatment of 0.04 and 0.08% methoprene on 5th instar nymphs, the THCs of the emerged males respectively exhibited 16.73, and 19.19% fall in their number.

Similarly, the affected females, emerged from treated 5th instar nymphs with 0.04 and 0.08% methoprene, showed slight reduction in THC by 3.11 and 4.93%. The females of untreated and acetone treated stock (control) contained 6088 ± 572.34 and 6394.5 ± 553.86 cells in 1 mm^3 of haemolymph.

The intermediates, which were formed by the application of higher concentrations (0.1 and 0.2%) of methoprene, respectively, contained 4000.5 ± 433.17 and 3960 ± 425.30 cells/mm^3 of haemolymph.

5.3 DHCs of 5th instar nymphs and adults of *Dysdercus cingulatus* affected with different concentrations of Methoprene.

Differential counts were recorded in the blood smears of nymphs after 6 hrs, 1 day, 3 days and 5 days following treatment with 0.04, 0.08, 0.1 and 0.2% methoprene. After adult emergence, percentages of each type of haemocytes were calculated in blood smears of one day old males and females. In addition to that, haemocyte profile of nymphal-adult intermediates resulted by two higher concentrations was also determined.

After 6 hrs (Table-30, Fig.16)

Prohaemocytes exhibited inconsistent increase in population which was statistically insignificant. Plasmatocytes were slightly increased by two lower concentration whereas at two higher concentrations they were, respectively, 3.6 and 5.86% less than the control. Adipohaemocytes showed concentration based linear reduction in population ($Y = 12.82 - 61.32\ X$, $r = -0.9085$, $P<0.001$). The highest concentration (0.2%) reduced the population of these haemocytes to 2.42% from 15.28% in control. Granulocytes underwent insignificant alterations in their proportion with respect to all the concentrations. Oenocytoids were almost same as in control.

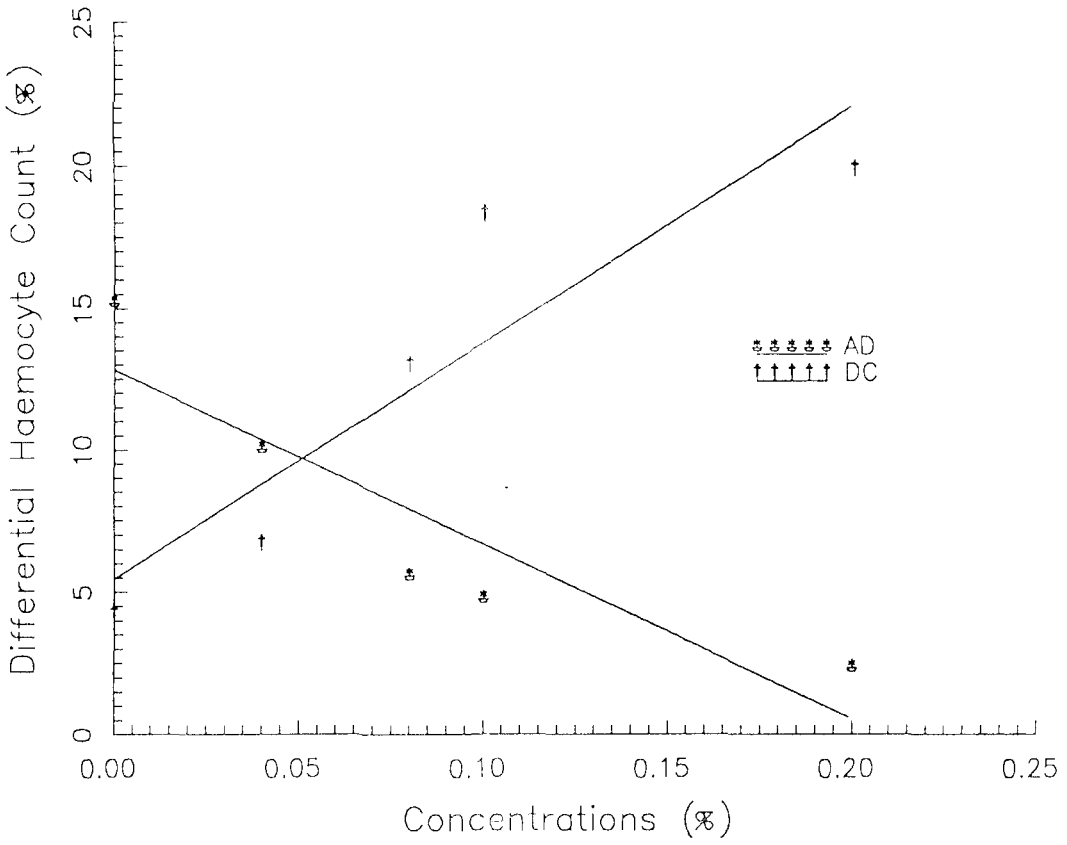

Fig. 16: Correlation between the Differential Haemocyte Count (%) and various concentrations of methoprene after 6 hrs of the treatment on one day old 5th instar nymphs of Dysdercus cingulatus.

The regression between concentration strength and damaged cell population yielded a positive linear correlation (Y = 5.45 + 83.55 X, r = 0.9124, P<0.001). The two higher concentrations increased these cells significantly (t=2.8264, and t= 4.0859, respectively P<0.05).

After 1 day (Table-31, Fig.17)

Prohaemocyte population showed an increasing trend following treatment with methoprene. Plasmatocytes exhibited variations in a narrow range in the treated batches compared to control. Adipohaemocytes were significantly low in the smears of treated nymphs with 0.08, 0.1 and 0.2% methoprene. Granulocytes underwent linear reduction following increasing concentrations of methoprene (Y = 8.9057 - 49.50 X, r = - 0.8456, P<0.001). On the other hand oenocytoid exhibited an increase in their relative proportion compared to control. The proportion of damaged cell was linearly increased with the increase in concentration of methoprene (Y = 06.82 + 117.0 X, r = 0.9604, P<0.001).

After 3 days (Table-32, Fig.18)

Prohaemocytes and plasmatocytes were proportionately more in the treated nymphs compared to control. Adipohaemocytes showed progressive linear reduction in population with increase in concentration of methoprene (Y = 28.66 - 132.15 X, r = - 0.9329, P<0.001). Granulocytes exhibited similar trend. Oenocytoids showed inconsistent trend whereas regression between damaged cells and increasing concentrations of methoprene yielded a positive correlation (Y = 6.911 + 91.83 X, r = 0.9233, P<0.001).

After 5 days (Table-33, Fig.19)

Prohaemocytes and plasmatocytes were proportionately more in population in the nymphs treated with all concentrations of methoprene. Adipohaemocytes showed linear reduction in relative percentage with respect to application of increasing concentrations (Y = 33.39 - 121.65 X, r = 0.9298, P<0.001). Granulocytes, although

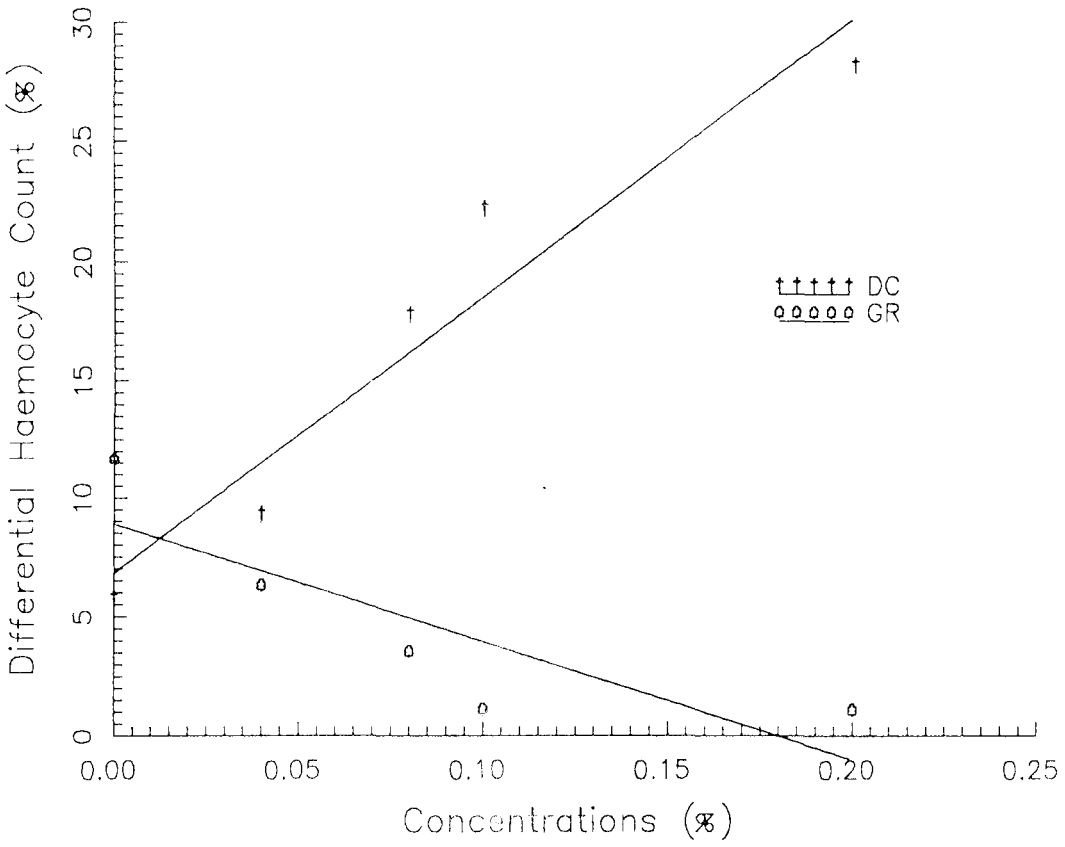

Fig. 17: Correlation between the Differential Haemocyte Count (%) and various concentrations of methoprene after 3 days of the treatment on one day old 5th instar nymphs of Dysdercus cingulatus.

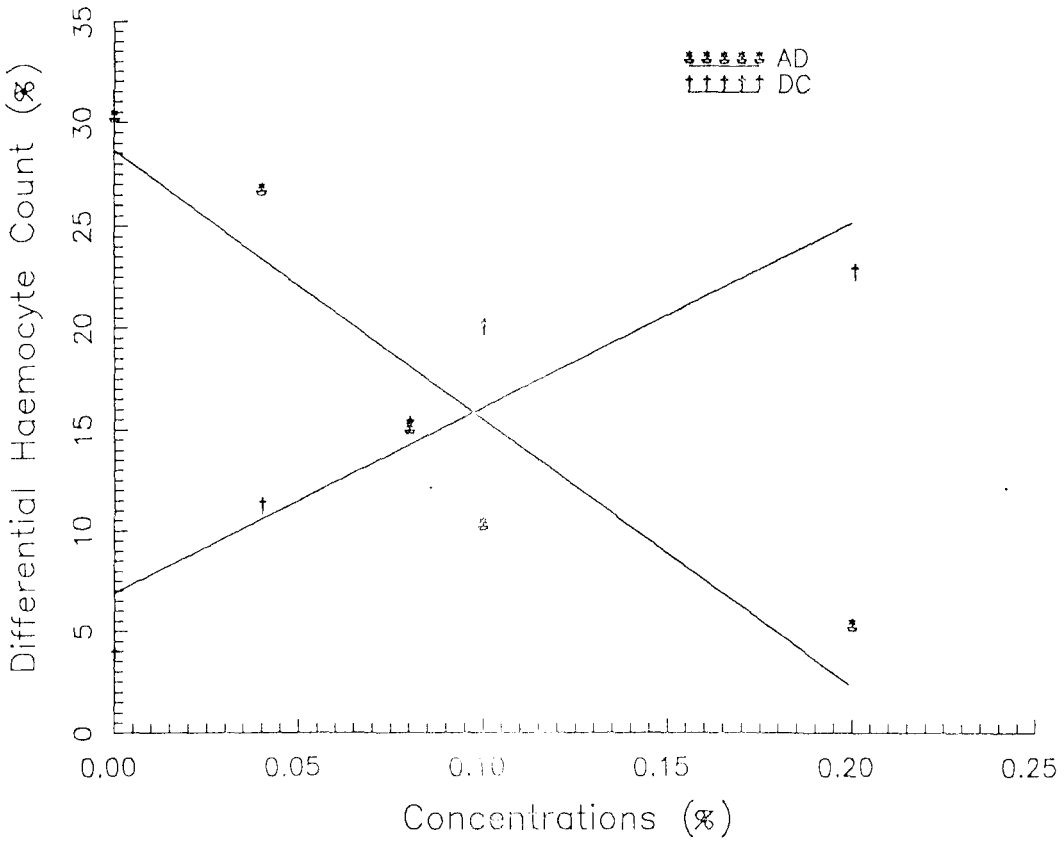

Fig. 18: Correlation between the Differential Haemocyte Count (%) and various concentrations of methoprene after 3 days of the treatment on one day old 5th instar nymphs of Dysdercus cingulatus.

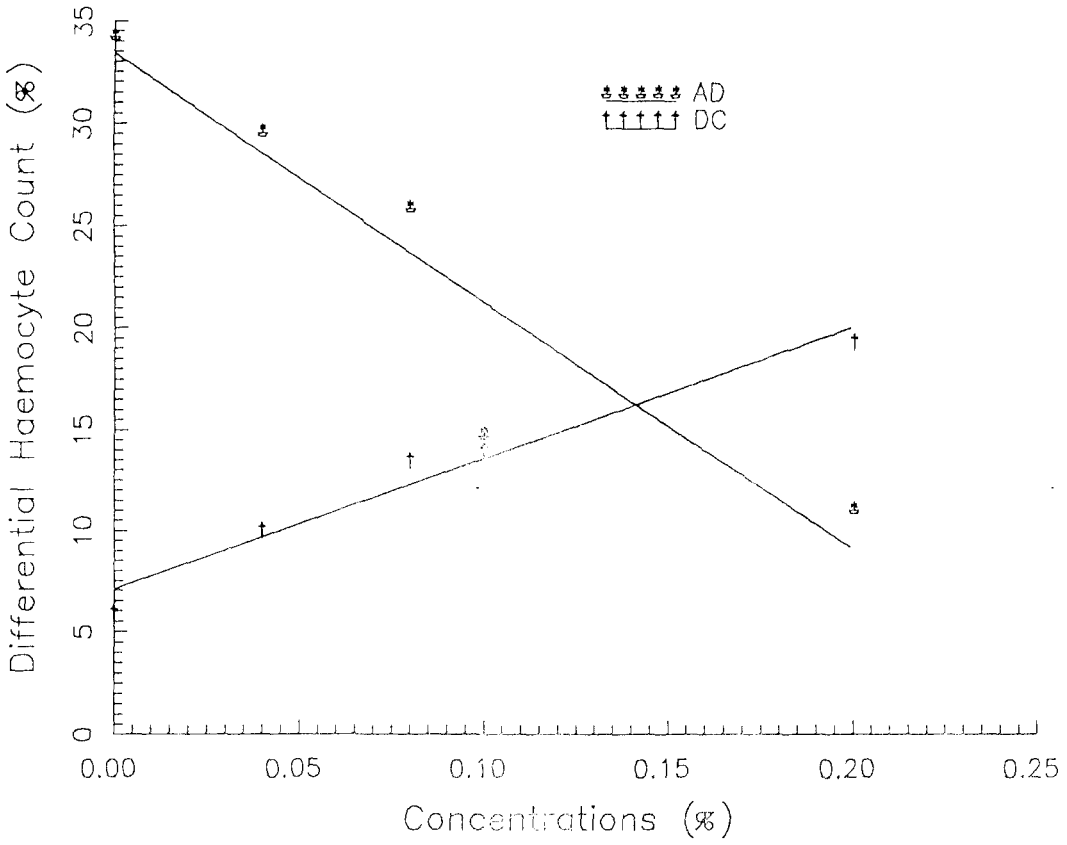

Fig. 19: Correlation between the Differential Haemocyte Count (%) and various concentrations of methoprene after 5 days of the treatment on one day old 5th instar nymphs of Dysdercus cingulatus.

exhibited reduction at all concentrations it was not concentration-dependent. Oenocytoids were proportionately slightly more compared to control. Population of damaged cells showed a concentration based positive linear correlation ($Y = 7.07 + 64.79 X$, $r = 0.9816$, $P<0.001$).

After imaginal ecdysis

When the treated nymphs moulted to adult stage, the lower concentrations caused a concentration based increase in the population of prohaemocytes and plasmatocytes which was statistically significant in case of former and insignificant in latter. Adipohaemocytes, on the other hand, showed a concentration based decrease compared to control. Granulocytes, however, were insignificantly increased at 0.04 and 0.08% methoprene in case of males whereas in the affected females these haemocytes underwent an insignificant reduction. Oenocytoids displayed an inconsistent trend in both the affected males and females. Damaged cells were comparatively more in the affected adult males (Table-34) and females (Table-35) at lower concentrations.

The intermediates, which emerged from nymphs treated with higher concentrations (0.1 and 0.2%) had 5.92 and 7.10% prohaemocytes. Plasmatocytes were major constituents of nymphal-adult intermediates. Adipohaemocytes were 25.84 and 22.60% of the total haemocytes. Granulocytes and oenocytoids constituted a small fraction of total population of haemocytes. Damaged cells constituted 15.38 and 15.22% of total cells in 0.1 and 0.2% methoprene affected insects, respectively.

Table 1: Total Haemocyte Counts determined at various time intervals following the topical application of various concentrations of Acephate on 1-2 day old 5th instar nymphs of *Dysdercus cingulatus*

Concentrations (%) 1 µl / nymph	Time Interval				Adult	
	6 Hrs. (Mean ±S. E)	1 Day (Mean ± S. E.)	3 Days (Mean ± S. E.)	5 Days (Mean ± S. E.)	Male (Mean ± S. E.)	Female (Mean ± S. E.)
Untreated	4630.5 ± 562.74	6457.5 ± 206.07	9274.5 ± 527.50	6435.0 ± 356.32	3528.0 ± 389.99	6372.0 ± 527.74
Solvent Treated	4536.0 ± 622.02	6592.5 ± 647.71	9180.0 ± 438.09	6669.0 ± 684.30	3532.5 ± 413.47	6448.5 ± 745.38
0.001	4702.5 ± 699057	6885.0 ± 702.81	8347.5 ± 404.19	6120.0 ± 411.39	3474.0 ± 628.94	6723.0 ± 380.09
0.002	4113.0 ± 652.24	5404.0 ± 559.95	7122.5 ± 386.97	5296.5 ± 543.21	2992.5 ± 383.56	5224.5 ± 717.27
0.004	2781.0 ± 491.18	3888.0 ± 417.22	3780.0 ± 395.19	4005.5 ± 505.29	2596.5 ± 414.12	4500.0 ± 611.32
0.006	2560.5 ± 472.04	3289.5 ± 496.55	2992.5 ± 406.43	2164.5 ± 331.09	--	--

Table 2: Total Haemocyte Counts determined at various time intervals following the topical application of various concentrations of Aminocarb on 1-2 day old 5th instar nymphs of *Dysdercus cingulatus*

Concentrations (%) 1 µl / nymph	Time Interval				Adult	
	6 Hrs. (Mean ± S. E.)	1 Day (Mean ± S. E.)	3 Days (Mean ± S. E.)	5 Days (Mean ± S. E.)	Male (Mean ± S. E.)	Female (Mean ± S. E.)
Untreated	4702.5 ± 499.23	6966.0 ± 246.84	10246.5± 530.57	6250.0 ± 334.14	3591.0 ± 230.18	6106.5 ± 499.66
Solvent Treated	4617.0 ± 1164.7	7218.0 ± 536.68	9679.0 ± 530.34	6502.5 ± 661.97	3384.0 ± 112.62	5949.0 ± 288.11
0.0025	5206.5 ± 465.62	7929.0 ± 539.51	7510.5 ± 639.78	6075.0 ± 704.00	3163.5 ± 278.55	5485.5 ± 490.55
0.004	4032.0 ± 416.55	4905.0 ± 959.12	4941.0 ± 1064.7	4729.5 ± 568.07	2750.5 ± 432.64	4941.0 ± 478.92
0.007	3132.0 ± 377.80	2884.5 ± 284.51	2718.0 ± 426.34	2295.0 ± 166.16	1989.0 ± 247.10	4540.5 ± 554.31
0.008	1926.0 ± 119.51	1822.5 ± 231.32	1773.0 ± 203.05	1462.5 ± 211.43	--	--

Table 3: Total Haemocyte Counts determined at various time intervals following the topical application of various concentrations of Cypermethrin 1-2 day old 5th instar nymphs of *Dysdercus cingulatus*

Concentrations (%) 1 µl / nymph	Time Interval				Adult	
	6 Hrs. (Mean ± S. E.)	1 Day (Mean ± S. E.)	3 Days (Mean ± S. E.)	5 Days (Mean ± S. E.)	Male (Mean ± S. E.)	Female (Mean ± S. E.)
Untreated	4729.5 ± 930.05	6916.5 ± 789.96	9121.5 ± 1255.5	6273.5 ± 616.86	3478.5 ± 496.31	6399.0 ± 756.07
Solvent Treated	4603.5 ± 656.04	6885.0 ± 674.70	9367.5 ± 1077.1	6376.5 ± 738.83	3361.5 ± 402.78	6210.0 ± 756.07
0.0004	4954.5 ± 819.09	7308.0 ± 280.70	8142.5 ± 1057.7	5890.5 ± 811.98	3177.0 ± 430.59	6169.5 ± 809.61
0.0006	4099.5 ± 278.07	4135.5 ± 445.78	7132.5 ± 1104.5	5179.5 ± 506.39	2934.0 ± 194.54	5391.0 ± 581.38
0.0008	2979.0 ± 318.25	3221.5 ± 766.72	3730.5 ± 482.59	4005.0 ± 506.37	2722.5 ± 173.26	4963.5 ± 344.37
0.001	1921.5 ± 142.00	2617.0 ± 402.03	2736.0 ± 530.14	2106.0 ± 269.96	--	--

Table 4: Total Haemocyte Counts determined at various time intervals following the topical application of various concentrations of Muristerone on 1-2 day old 5th instar nymphs of *Dysdercus cingulatus*

Concentrations (%) 1 µl / nymph	Time Interval				Adult	
	6 Hrs. (Mean ± S. E.)	1 Day (Mean ± S. E.)	3 Days (Mean ± S. E.)	5 Days (Mean ± S. E.)	Male (Mean ± S. E.)	Female (Mean ± S. E.)
Untreated	4549.5 ± 378.67	6988.5 ± 773.57	9531.0 ± 652.91	6075.0 ± 768.73	3663.0 ± 611.17	6300.0 ± 505.22
Solvent Treated	4851.0 ± 788.13	7038.0 ± 346.61	9688.5 ± 440.02	6228.0 ± 362.76	3649.5 ± 593.39	6574.5 ± 597.81
0.2	5044.5 ± 506.25	7915.5 ± 813.63	11124 ± 734.60	5616.0 ± 781.10	4261.5 ± 413.88	6916.5 ± 298.31
0.4	5314.5 ± 440.99	5922.0 ± 360.80	12748.5 ± 1332.4	4936.5 ± 452.94	4536.0 ± 649.06	7303.5 ± 556.74
0.6	3226.5 ± 366.59	4248.0 ± 596.03	6033.0 ± 815.93	--	--	--
0.8	2875.5 ± 424.61	3348.0 ± 529.49	4095.0 ± 502.16	--	--	--

Table 5: Total Haemocyte Counts determined at various time intervals following the topical application of various concentrations of Methoprene on 1-2 day old 5^{th} instar nymphs of *Dysdercus cingulatus*

Concentrations (%) 1 µl / nymph	Time Interval				Adult	
	6 Hrs. (Mean ± S. E.)	1 Day (Mean ± S. E.)	3 Days (Mean ± S. E.)	5 Days (Mean ± S. E.)	Male (Mean ± S. E.)	Female (Mean ± S. E.)
Untreated	4405.5 ± 285.53	6097.5 ± 514.87	9360.0 ± 436.06	5958.0 ± 400.94	3560.5 ± 384.05	6088.5 ± 572.34
Solvent Treated	4800.0 ± 431.59	6232.5 ± 431.98	9500.0 ± 455.76	6300.0 ± 214.05	3775.5 ± 208.58	6394.5 ± 553.86
0.04	4887.0 ± 505.29	6111.5 ± 593.32	9184.5 ± 403.65	6201.0 ± 610.06	3370.5 ± 487.29	6195.5 ± 569.15
0.08	5130.0 ± 386.52	6398.5 ± 562.15	8176.5 ± 409.27	5508.0 ± 312.45	3051.0 ± 213.64	6079.5 ± 402.83
0.1	4000.0 ± 345.84	5814.0 ± 434.29	6997.5 ± 560.11	5220.0 ± 367.72		
0.2	3294.0 ± 430.13	5049.0 ± 320.31	6016.5 ± 206.78	4734.0 ± 229.74	--	--

Table 6: Differential Haemocyte Counts determined after 6 hrs. following the topical application of various concentrations of Acephat on 1-2 day old 5th instar nymphs of *Dysdercus cingulatus*

Concentrations (%) 1 µl / nymph	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Adipohaemocytes % ± S. E.	Granulocytes % ± S. E.	Oenocytoids % ± S. E.	Disintegrated Cells % ± S. E.
Untreated	09.08 ± 0.85	53.02 ± 5.15	16.58 ± 3.23	08.50 ± 1.15	04.00 ± 1.47	08.92 ± 2.55
Solvent Treated	09.26 ± 1.16	53.20 ± 4.32	17.06± 3.51	10.44 ± 3.07	03.16 ± 1.19	06.64 ± 1.69
0.001	18.18 ± 2.06	36.78 ± 4.39	15.84± 1.47	09.92 ± 0.81	05.94 ± 1.19	13.34 ± 3.00
0.002	18.80 ± 2.08	34.96 ± 3.56	11.32 ± 1.58	08.68 ± 0.56	06.96 ± 0.97	19.92 ± 2.23
0.004	23.46 ± 2.60	22.58 ± 3.00	09.72 ± 2.30	07.72 ± 1.61	12.60 ± 1.70	23.72 ± 2.39
0.006	27.16 ± 2.26	08.86 ± 1.78	00.00	05.36 ± 1.46	12.90 ± 0.60	45.62 ± 2.37

Table 7: Differential Haemocyte Counts determined after 1 day following the topical application of various concentrations of Acephate on 1-2 day old 5th instar nymphs of *Dysdercus cingulatus*

Concentrations (%) 1 µl / nymph	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Adipohaemocytes % ± S. E.	Granulocytes % ± S. E.	Oenocytoids % ± S. E.	Disintegrated Cells % ± S. E.
Untreated	11.30 ± 2.19	47.12 ± 5.16	23.62 ± 4.08	06.56 ± 1.14	03.60 ± 1.30	07.88 ± 1.94
Solvent Treated	11.96 ± 2.15	43.62 ± 2.48	22.14 ± 3.34	8.42 ± 0.87	04.12 ± 0.99	09.72 ± 1.64
0.001	22.02 ± 2.05	23.30 ± 1.31	21.14 ± 2.42	15.32 ± 2.23	07.30 ± 1.04	10.76 ± 1.51
0.002	25.72 ± 2.48	10.08 ± 2.86	15.10 ± 3.28	12.84 ± 1.56	09.14 ± 0.87	17.98 ± 1.92
0.004	27.34 ± 4.54	15.70 ± 2.16	01.28 ± 0.79	05.62 ± 1.49	11.18 ± 0.66	38.60 ± 2.81
0.006	28.88 ± 1.89	07.26 ± 1.42	00.00	01.74 ± 0.37	15.58 ± 2.59	47.56 ± 3.21

Table 8: Differential Haemocyte Counts determined after 3 days following the topical application of various concentrations of Acephate on 1-2 day old 5th instar nymphs of *Dysdercus cingulatus*

Concentrations (%) 1 µl / nymph	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Adipohaemocytes % ± S. E.	Granulocytes % ± S. E.	Oenocytoids % ± S. E.	Disintegrated Cells % ± S. E.
Untreated	15.48 ± 1.55	32.78 ± 2.74	31.12 ± 4.15	10.84 ± 1.78	01.98 ± 1.07	07.68 ± 1.34
Solvent Treated	17.00 ± 2.55	33.78 ± 2.74	30.34 ± 2.74	06.64 ± 1.25	03.72 ± 0.94	08.78 ± 1.27
0.001	24.26 ± 2.05	24.58 ± 1.39	20.12 ± 2.03	14.08 ± 1.47	05.90 ± 1.27	10.92 ± 2.25
0.002	34.56 ± 1.95	14.34 ± 3.42	10.76 ± 1.16	14.18 ± 1.89	10.08 ± 1.15	16.10 ± 1.52
0.004	18.74 ± 4.36	13.00 ± 2.81	00.00	18.18 ± 1.22	15.42 ± 2.00	34.66 ± 2.78
0.006	30.84 ± 2.85	10.70 ± 2.27	00.00	00.00	17.50 ± 1.87	40.90 ± 3.48

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Table 9: Differential Haemocyte Counts determined after 5 days following the topical application of various concentrations of Acephate on 1-2 day old 5th instar nymphs of *Dysdercus cingulatus*

Concentrations (%) 1 µl / nymph	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Adipohaemocytes % ± S. E.	Granulocytes % ± S. E.	Oenocytoids % ± S. E.	Disintegrated Cells % ± S. E.
Untreated	06.30 ± 1.97	32.21 ± 4.63	39.18 ± 2.60	13.44 ± 1.87	04.26 ± 0.80	05.34 ± 0.99
Solvent Treated	06.08 ± 1.17	35.82 ± 2.61	31.64 ± 2.39	15.92 ± 1.75	03.78 ± 0.63	06.82 ± 1.57
0.001	17.06 ± 3.30	25.16 ± 2.77	23.60 ± 5.34	12.68 ± 1.73	07.44 ± 1.89	07.30 ± 1.30
0.002	22.36 ± 4.54	21.54 ± 1.19	19.52 ± 0.59	11.66 ± 1.34	08.30 ± 1.11	16.64 ± 2.22
0.004	29.58 ± 3.13	14.10 ± 1.62	00.00	00.00	14.46 ± 3.70	41.92 ± 3.19
0.006	25.64 ± 2.45	02.62 ± 1.87	00.00	00.00	16.48 ± 2.81	47.08 ± 7.27

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Table 10: Differential Haemocyte Counts determined in one day old adult male following the topical application of various concentrations of Acephate on 1-2 day old 5th instar nymphs of *Dysdercus cingulatus*

Concentrations (%) 1 µl / nymph	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Adipohaemocytes % ± S. E.	Granulocytes % ± S. E.	Oenocytoids % ± S. E.	Disintegrated Cells % ± S. E.
Untreated	02.48 ± 1.13	39.66 ± 5.25	45.30 ± 4.84	06.76 ± 1.53	02.20 ± 0.62	03.56 ± 0.71
Solvent Treated	2.86 ± 0.80	37.32 ± 4.23	44.34 ± 5.02	07.20 ± 1.91	01.36 ± 0.49	05.92 ± 2.39
0.001	03.20 ± 0.45	31.92 ± 2.84	42.74 ± 4.02	06.78 ± 1.63	05.44 ± 1.29	09.92 ± 2.51
0.002	07.86 ± 1.65	28.58 ± 3.71	33.06 ± 4.74	09.98 ± 1.60	04.74 ± 0.94	13.80 ± 1.61
0.004	15.10 ± 2.81	31.84 ± 5.82	33.84 ± 5.18	07.26 ± 2.26	01.58 ± 0.77	10.44 ± 1.10
0.006	00.00	00.00	00.00	00.00	00.00	00.00

Table 11: Differential Haemocyte Counts determined in one day old adult females following the topical application of various concentrations of Acephate on 1-2 day old 5th instar nymphs of *Dysdercus cingulatus*

Concentrations (%) 1 µl / nymph	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Adipohaemocytes % ± S. E.	Granulocytes % ± S. E.	Oenocytoids % ± S. E.	Disintegrated Cells % ± S. E.
Untreated	05.78 ± 1.77	50.96 ± 2.11	28.54 ± 3.41	06.30 ± 1.74	01.14 ± 0.47	07.36 ± 1.70
Solvent Treated	05.05 ± 1.72	50.52 ± 5.93	28.18 ± 3.78	06.64 ± 1.61	01.82 ± 0.64	05.74 ± 1.31
0.001	07.88 ± 0.66	52.52 ± 2.09	22.26 ± 3.00	07.52 ± 1.24	01.86 ± 0.26	07.94 ± 1.28
0.002	13.98 ± 7.56	26.74 ± 1.98	25.20 ± 2.89	09.68 ± 0.72	02.62 ± 0.80	10.20 ± 1.49
0.004	18.52 ± 1.44	32.40 ± 1.68	23.40 ± 1.14	11.06 ± 0.60	02.98 ± 0.75	11.80 ± 0.89
0.006	00.00	00.00	00.00	00.00	00.00	00.00

Table 12: Differential Haemocyte Counts determined after 6 hrs. following the topical application of various concentrations of Aminocarb on 1-2 day old 5th instar nymphs of *Dysdercus cingulatus*

Concentrations (%) 1 µ l / nymph	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Adipohaemocytes % ± S. E.	Granulocytes % ± S. E.	Oenocytoids % ± S. E.	Disintegrated Cells % ± S. E.
Untreated	11.72 ± 1.99	48.50 ± 3.00	17.96 ± 2.11	14.32 ± 1.96	03.46 ± 0.54	04.08 ± 0.60
Solvent Treated	10.84 ± 1.92	52.06 ± 3.64	19.10 ± 3.23	12.34 ± 2.09	02.36 ± 0.71	03.34 ± 1.03
0.0025	18.94 ± 1.24	41.28 ± 3.31	13.18 ± 2.26	12.92 ± 2.44	02.70 ± 0.57	11.14 ± 2.07
0.004	22.20 ± 2.74	26.56 ± 2.47	10.38 ± 1.91	15.38 ± 2.04	07.04 ± 0.98	18.44 ± 1.84
0.007	29.74 ± 4.62	18.70 ± 2.64	06.28 ± 2.26	08.66 ± 1.11	11.98 ± 4.01	24.48 ± 5.93
0.008	24.96 ± 3.51	09.02 ± 1.56	00.72 ± 0.31	02.58 ± 0.44	16.88 ± 2.68	46.8 ± 3.05

Table 13: Differential Haemocyte Counts determined after 1 day following the topical application of various concentrations of Aminocarb on 1-2 day old 5th instar nymphs of *Dysdercus cingulatus*

Concentrations (%) 1 µl / nymph	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Adipohaemocytes % ± S. E.	Granulocytes % ± S. E.	Oenocytoids % ± S. E.	Disintegrated Cells % ± S. E.
Untreated	12.08 ± 1.58	48.28 ± 3.23	24.08 ± 1.99	08.28 ± 1.38	02.66 ± 1.17	04.60 ± 0.94
Solvent Treated	12.74 ± 1.98	49.32 ± 1.66	23.20 ± 1.72	08.42 ± 1.46	02.60 ± 0.58	03.80 ± 0.39
0.0025	19.08 ± 1.11	29.54 ± 2.72	18.16 ± 1.27	19.80 ± 1.79	03.74 ± 0.76	09.42 ± 1.40
0.004	30.80 ± 1.83	23.46 ± 2.81	11.62 ± 2.09	09.78 ± 1.26	06.44 ± 1.04	17.94 ± 2.23
0.007	22.64 ± 2.05	16.98 ± 1.13	04.26 ± 1.54	08.38 ± 0.45	11.84 ± 2.26	35.86 ± 3.30
0.008	14.22 ± 2.02	11.34 ± 1.78	00.00	00.00	23.00 ± 2.47	51.28 ± 2.74

Table 14: Differential Haemocyte Counts determined after 3 days following the topical application of various concentrations of Aminocarb on 1-2 day old 5th instar nymphs of *Dysdercus cingulatus*

Concentrations (%) 1 µl / nymph	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Adipohaemocytes % ± S. E.	Granulocytes % ± S. E.	Oenocytoids % ± S. E.	Disintegrated Cells % ± S. E.
Untreated	15.78 ± 3.15	35.90 ± 4.91	29.92 ± 3.03	10.00 ± 1.34	03.42 ± 0.64	07.98 ± 1.28
Solvent Treated	15.76 ± 3.51	33.26 ± 3.30	30.80 ± 1.82	11.14 ± 1.29	03.44 ± 1.00	05.58 ± 1.92
0.0025	18.24 ± 1.63	39.84 ± 3.59	23.24 ± 1.69	10.36 ± 1.60	03.98 ± 1.11	05.42 ± 1.33
0.004	20.00 ± 1.79	28.78 ± 1.42	10.28 ± 1.50	19.04 ± 1.19	08.58 ± 1.27	13.14 ± 0.90
0.007	26.48 ± 1.77	24.56 ± 2.09	00.00	09.38 ± 1.46	09.38 ± 1.40	30.00 ± 2.19
0.008	21.24 ± 2.69	23.30 ± 2.62	00.00	00.00	11.52 ± 0.92	43.94 ± 4.15

Table 15: Differential Haemocyte Counts determined after 5 days following the topical application of various concentrations of Aminocarb on 1-2 day old 5th instar nymphs of *Dysdercus cingulatus*

Concentrations (%) 1 µl / nymph	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Adipohaemocytes % ± S. E.	Granulocytes % ± S. E.	Oenocytoids % ± S. E.	Disintegrated Cells % ± S. E.
Untreated	06.52 ± 0.58	34.30 ± 3.80	37.54 ± 3.76	12.16 ± 1.14	03.92 ± 0.91	05.72 ± 0.97
Solvent Treated	06.98 ± 0.53	37.88 ± 2.57	31.16 ± 1.71	14.18 ± 1.98	03.86 ± 0.42	05.76 ± 0.36
0.0025	19.48 ± 2.96	31.84 ± 2.62	18.74 ± 2.77	09.90 ± 0.21	09.88 ± 1.89	10.14 ± 1.11
0.004	32.44 ± 6.49	21.88 ± 2.86	09.50 ± 1.07	10.78 ± 0.47	06.74 ± 0.64	15.24 ± 2.50
0.007	37.76 ± 5.45	09.80 ± 1.56	00.00	10.30 ± 0.85	15.38 ± 1.75	35.72 ± 4.75
0.008	29.32 ± 2.60	07.58 ± 1.38	00.00	00.00	19.08 ± 2.09	44.04 ± 2.60

Table 16: Differential Haemocyte Counts determined in adult male following the topical application of various concentrations of Aminocarb on 1-2 day old 5th instar nymphs of *Dysdercus cingulatus*

Concentrations (%) 1 µl / nymph	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Adipohaemocytes % ± S. E.	Granulocytes % ± S. E.	Oenocytoids % ± S. E.	Disintegrated Cells % ± S. E.
Untreated	03.12 ± 0.70	40.00 ± 1.98	43.98 ± 2.75	71.80 ± 1.22	01.82 ± 0.52	05.08 ± 0.88
Solvent Treated	03.68 ± 0.26	39.38 ± 2.22	41.76 ± 1.73	06.82 ± 0.83	02.22 ± 0.39	06.16 ± 1.54
0.0025	04.16 ± 0.52	42.76 ± 2.17	38.92 ± 2.17	04.12 ± 0.95	02.16 ± 0.68	08.04 ± 0.84
0.004	04.12 ± 0.55	39.16 ± 2.95	38.24 ± 2.83	07.20 ± 0.62	06.32 ± 1.26	05.92 ± 1.08
0.007	13.10 ± 1.22	33.20 ± 2.39	31.50 ± 2.04	06.76 ± 1.20	06.06 ± 0.61	09.46 ± 0.96
0.008	00.00	00.00	00.00	00.00	00.00	00.00

Table 17: Differential Haemocyte Counts determined in adult female following the topical application of various concentrations of Aminocarb on 1-2 day old 5th instar nymphs of *Dysdercus cingulatus*

Concentrations (%) 1 µl / nymph	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Adipohaemocytes % ± S. E.	Granulocytes % ± S. E.	Oenocytoids % ± S. E.	Disintegrated Cells % ± S. E.
Untreated	05.22 ± 0.38	50.62 ± 3.80	26.18 ± 2.28	07.16 ± 1.54	04.68 ± 0.99	06.00 ± 1.09
Solvent Treated	05.30 ± 0.42	50.26 ± 2.13	24.26 ± 2.35	07.98 ± 0.94	03.70 ± 0.26	08.64 ± 0.88
0.0025	10.54 ± 1.36	36.00 ± 4.01	27.86± 2.92	07.84 ± 1.26	05.52 ± 1.10	12.08 ± 1.91
0.004	17.14 ± 2.81	35.00 ± 2.69	22.04 ± 2.44	07.80 ± 1.16	04.38 ± 1.29	13.64 ± 1.65
0.007	16.28 ± 1.54	31.00 ± 2.37	18.78 ± 1.48	12.84 ± 1.95	05.08 ± 1.11	16.02 ± 1.42
0.008	00.00	00.00	00.00	00.00	00.00	00.00

Table 18: Differential Haemocyte Counts determined after 6 hrs. following the topical application of various concentrations of Cypermethrin on 1-2 day old 5th instar nymphs of *Dysdercus cingulatus*

Concentrations (%) 1 µl / nymph	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Adipohaemocytes % ± S. E.	Granulocytes % ± S. E.	Oenocytoids % ± S. E.	Disintegrated Cells % ± S. E.
Untreated	10.80 ± 1.75	44.68 ± 4.67	19.17 ± 2.44	12.70 ± 2.60	04.02 ± 1.02	08.64 ± 1.42
Solvent Treated	09.20 ± 1.34	47.38 ± 5.24	15.14 ± 2.54	14.98 ± 2.66	04.18 ± 1.16	06.90 ± 1.18
0.0004	11.30 ± 2.11	42.00 ± 2.94	10.00 ± 2.25	16.36 ± 2.22	07.10 ± 1.12	13.32 ± 2.01
0.0006	27.06 ± 1.81	25.70 ± 2.94	10.12 ± 1.10	07.96 ± 1.15	07.48 ± 1.00	21.64 ± 0.65
0.0008	31.32 ± 1.81	27.72 ± 2.41	00.00	00.00	15.04 ± 1.70	25.72 ± 2.25
0.001	33.08 ± 3.35	07.52 ± 1.35	00.00	00.00	16.70 ± 1.84	43.12 ± 3.33

Table 19: Differential Haemocyte Counts determined after 1 day following the topical application of various concentrations of Cypermethrin in 1-2 day old 5th instar nymphs of *Dysdercus cingulatus*

Concentrations (%) 1 µ l / nymph	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Adipohaemocytes % ± S. E.	Granulocytes % ± S. E.	Oenocytoids % ± S. E.	Disintegrated Cells % ± S. E.
Untreated	14.72 ± 2.14	46.74 ± 4.07	22.02 ± 3.21	10.46 ± 1.59	04.06 ± 1.31	05.58 ± 1.47
Solvent Treated	14.54 ± 1.71	43.32 ± 3.78	43.32 ± 3.78	10.50 ± 1.92	04.82 ± 1.10	07.28 ± 2.51
0.0004	23.68 ± 4.40	27.22 ± 3.02	15.20 ± 2.27	17.26 ± 2.27	04.98 ± 0.89	11.80 ± 1.27
0.0006	21.20 ± 2.74	28.36 ± 2.84	08.80 ± 2.12	09.82 ± 0.84	10.90 ± 2.70	20.88 ± 2.71
0.0008	26.44 ± 2.48	11.32 ± 1.86	02.56 ± 0.60	02.30 ± 0.43	06.16 ± 2.44	41.48 ± 1.24
0.001	25.24 ± 2.24	04.66 ± 1.26	00.00	00.00	17.36 ± 2.67	48.78 ± 5.52

Table 20: Differential Haemocyte Counts determined after 3 days following the topical application of various concentrations of Cypermethrin on 1-2 day old 5th instar nymphs of *Dysdercus cingulatus*

Concentrations (%) 1 µl / nymph	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Adipohaemocytes % ± S. E.	Granulocytes % ± S. E.	Oenocytoids % ± S. E.	Disintegrated Cells % ± S. E.
Untreated	15.06 ± 1.95	35.70 ± 2.47	26.36 ± 1.93	10.18 ± 1.78	04.56 ± 0.84	07.98 ± 1.17
Solvent Treated	15.98 ± 2.08	34.14 ± 3.12	27.28 ± 1.75	11.96 ± 1.37	04.28 ± 0.66	04.66 ± 2.03
0.0004	16.82 ± 1.62	27.42 ± 2.49	21.52 ± 2.03	18.58 ± 2.08	05.20 ± 1.13	10.52 ± 2.72
0.0006	23.60 ± 1.69	27.62 ± 3.26	06.88 ± 1.34	12.84 ± 1.32	09.64 ± 1.48	19.34 ± 1.27
0.0008	26.52 ± 3.96	25.46 ± 3.86	00.00	02.10 ± 0.35	11.34 ± 1.17	34.58 ± 1.66
0.001	20.10 ± 3.21	17.94 ± 3.41	00.00	00.00	12.50 ± 2.85	49.46 ± 2.96

Table 21: Differential Haemocyte Counts determined after 5 days following the topical application of various c Cypermethrin on 1-2 day old 5th instar nymphs of *Dysdercus cingulatus*

Concentrations (%) 1 µl / nymph	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Adipohaemocytes % ± S. E.	Granulocytes % ± S. E.	Oenocytoids % ± S. E.	Disintegrat % ± S
Untreated	04.44 ± 0.60	39.16 ± 3.75	29.82 ± 3.64	14.68 ± 1.96	03.84 ± 0.30	08.06 ±
Solvent Treated	05.54 ± 0.85	39.80 ± 2.80	29.02 ± 2.88	13.46 ± 1.62	04.92 ± 1.14	07.28 ±
0.0004	11.62 ± 1.75	27.36 ± 4.49	24.18 ± 2.52	16.00 ± 1.75	04.88 ± 0.78	15.80 ±
0.0006	28.08 ± 1.96	25.08 ± 2.30	09.16 ± 0.81	08.12 ± 0.62	09.56 ± 1.68	18.08 ±
0.0008	32.96 ± 3.70	11.46 ± 1.08	00.00	00.00	12.38 ± 1.11	43.20 ±
0.001	00.00	00.00	00.00	00.00	00.00	00.0

Table 22: Differential Haemocyte Counts determined in one day old adult male following the topical application of various concentrations of Cypermethrin on 1-2 day old 5th instar nymphs of *Dysdercus cingulatus*

Concentrations %	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Adipohaemocytes % ± S. E.	Granulocytes % ± S. E.	Oenocytoids % ± S. E.	Disintegrated Cells % ± S. E.
Untreated	05.32 ± 0.81	37.80 ± 1.92	39.80 ± 2.83	07.94 ± 0.80	02.54 ± 0.94	06.62 ± 0.82
Solvent Treated	05.38 ± 1.06	36.76 ± 2.23	42.12± 2.83	07.12 ± 0.68	03.06 ± 0.53	05.58 ± 1.19
0.0004	06.12 ± 1.06	38.96 ± 1.25	29.44 ± 3.70	10.86 ± 2.00	05.72 ± 1.26	12.28 ± 1.78
0.0006	09.74 ± 0.94	33.00 ± 3.37	24.72 ± 2.69	12.56 ± 0.94	07.26 ± 1.00	12.62 ± 1.23
0.0008	09.94 ± 0.57	32.04 ± 1.46	17.34 ± 3.32	16.04 ± 1.50	08.38 ± 1.15	16.26 ± 0.96
0.001	00.00	00.00	00.00	00.00	00.00	00.00

Table 23: Differential Haemocyte Counts determined in one day old adult female following the topical application of various concentrations of Cypermethrin on 1-2 day old 5th instar nymphs of *Dysdercus cingulatus*

Concentrations (%) 1 µl / nymph	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Adipohaemocytes % ± S. E.	Granulocytes % ± S. E.	Oenocytoids % ± S. E.	Disintegrated Cells % ± S. E.
Untreated	06.30 ± 1.37	49.16 ± 7.67	28.44 ± 3.31	07.67 ± 0.90	02.84 ± 0.89	05.74 ± 1.16
Solvent Treated	06.80 ± 0.99	43.96 ± 2.39	28.98 ± 1.58	10.40 ± 0.97	03.42 ± 0.76	06.40 ± 0.86
0.0004	06.42 ± 1.13	50.56 ± 2.97	19.78± 2.28	06.16 ± 0.45	05.20 ± 0.68	11.80 ± 1.78
0.0006	10.72 ± 0.96	40.60 ± 2.92	18.68 ± 2.33	09.88 ± 1.38	05.44 ± 1.13	14.88 ± 3.37
0.0008	15.38 ± 1.65	32.46 ± 3.62	17.64 ± 2.02	07.62 ± 1.59	07.50 ± 0.90	19.52 ± 2.20
0.001	00.00	00.00	00.00	00.00	00.00	00.00

Table 24: Differential Haemocyte Counts determined after 6 hrs. following the topical application of various concentrations of Muristerone on 1-2 day old 5th instar nymphs of *Dysdercus cingulatus*

Concentrations (%) 1 µl / nymph	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Adipohaemocytes % ± S. E.	Granulocytes % ± S. E.	Oenocytoids % ± S. E.	Disintegrated Cells % ± S. E.
Untreated	10.44 ± 1.51	51.54 ± 3.61	16.30 ± 1.56	11.88 ± 2.43	03.38 ± 0.50	06.04 ± 1.15
Solvent Treated	09.66 ± 0.85	52.60 ± 2.80	14.50± 1.70	15.84 ± 3.09	02.50 ± 0.48	04.80 ± 1.08
0.2	06.36 ± 0.51	55.02 ± 2.96	19.84± 3.18	07.62 ± 1.43	02.76 ± 0.56	08.34 ± 1.59
0.4	05.66 ± 1.00	50.86 ± 2.34	21.56 ± 2.35	05.62 ± 1.09	02.92 ± 0.46	13.30 ± 2.24
0.6	04.66 ± 0.64	42.04 ± 4.49	23.24 ± 2.18	06.56 ± 0.85	03.54 ± 0.52	20.02 ± 2.29
0.8	10.30 ± 1.05	50.26 ± 3.41	07.94 ± 0.70	05.78 ± 0.87	04.42 ± 1.08	21.06 ± 2.18

Table 25: Differential Haemocyte Counts determined after one day following the topical application of various concentrations of Muristerone on 1-2 day old 5th instar nymphs of *Dysdercus cingulatus*

Concentrations (%) 1 µl / nymph	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Adipohaemocytes % ± S. E.	Granulocytes % ± S. E.	Oenocytoids % ± S. E.	Disintegrated Cells % ± S. E.
Untreated	12.32 ± 0.93	45.92 ± 2.51	21.76 ± 1.82	09.40 ± 2.31	03.84 ± 0.90	06.72 ± 0.68
Solvent Treated	10.66 ± 0.91	45.92 ± 2.65	20.78 ± 1.76	11.38 ± 1.70	04.00 ± 0.42	07.40 ± 1.44
0.2	06.82 ± 0.91	53.30 ± 3.23	21.14 ± 2.89	05.82 ± 1.28	02.94 ± 0.67	10.28 ± 2.30
0.4	09.20 ± 1.01	50.58 ± 1.72	15.10 ± 1.62	06.36 ± 1.41	03.92 ± 0.72	14.80 ± 1.97
0.6	09.26 ± 2.01	51.82 ± 3.38	11.46 ± 1.71	03.70 ± 0.67	02.94 ± 0.91	20.94 ± 2.68
0.8	09.30 ± 1.30	47.56 ± 2.94	09.8 ± 1.13	04.82 ± 0.90	03.18 ± 0.49	26.00 ± 3.52

Table 26: Differential Haemocyte Counts determined after 3 days following the topical application of various concentrations of Muristerone on 1-2 day old 5th instar nymphs of *Dysdercus cingulatus*

Concentrations (%) 1 µl / nymph	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Adipohaemocytes % ± S. E.	Granulocytes % ± S. E.	Oenocytoids % ± S. E.	Disintegrated Cells % ± S. E.
Untreated	15.94 ± 1.37	32.80 ± 3.58	31.14 ± 4.02	09.94 ± 1.11	04.78 ± 0.64	05.48 ± 1.31
Solvent Treated	13.66 ± 1.75	33.60 ± 1.62	30.60± 2.09	12.10 ± 1.97	02.56 ± 0.58	08.60 ± 0.84
0.2	08.26 ± 0.53	37.34 ± 2.07	27.52± 2.79	13.34 ± 2.08	03.80 ± 0.67	09.80 ± 0.71
0.4	14.92 ± 1.91	32.70 ± 1.86	25.42 ± 2.26	07.10 ± 0.88	03.08 ± 1.08	16.78 ± 1.50
0.6	15.70 ± 1.50	35.60 ± 4.56	20.80 ± 2.09	02.84 ± 0.38	02.46 ± 0.40	22.68 ± 3.77
0.8	16.36 ± 2.01	40.08 ± 2.88	11.6 ± 1.41	01.82 ± 1.51	03.96 ± 0.92	26.52 ± 3.20

Table 27: Differential Haemocyte Counts determined after 5 days following the topical application of various concentrations of Muristerone on 1-2 day old 5th instar nymphs of *Dysdercus cingulatus*

Concentrations (%) 1 µl / nymph	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Adipohaemocytes % ± S. E.	Granulocytes % ± S. E.	Oenocytoids % ± S. E.	Disintegrated Cells % ± S. E.
Untreated	07.06 ± 0.60	35.84 ± 3.10	33.06 ± 4.75	09.84 ± 0.92	05.58 ± 0.93	06.82 ± 0.83
Solvent Treated	06.24 ± 0.86	33.06 ± 2.20	35.62 ± 2.78	12.06 ± 0.97	05.32 ± 0.70	07.72 ± 0.85
0.2	08.16 ± 0.65	37.24 ± 2.85	30.62 ± 1.82	10.12 ± 0.08	04.54 ± 0.69	09.32 ± 0.64
0.4	08.78 ± 0.88	32.22 ± 2.18	30.04 ± 3.90	12.08 ± 1.09	06.14 ± 0.65	10.76 ± 2.42
0.6	-	-	-	-	-	-
0.8	-	-	-	-	-	-

Table 28: Differential Haemocyte Counts determined in adult male following the topical application of various concentrations of Muristerone on 1-2 day old 5th instar nymphs of *Dysdercus cingulatus*

Concentrations (%) 1 µl / nymph	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Adipohaemocytes % ± S. E.	Granulocytes % ± S. E.	Oenocytoids % ± S. E.	Disintegrated Cells % ± S. E.
Untreated	02.96 ± 0.48	37.68 ± 2.58	45.48 ± 1.59	07.32 ± 2.22	01.88 ± 0.69	04.62 ± 1.98
Solvent Treated	02.94 ± 0.41	37.40 ± 1.44	43.02 ± 4.65	05.66 ± 0.48	03.54 ± 0.86	04.20 ± 1.48
0.2	12.24 ± 1.48	45.04 ± 2.52	20.14 ± 1.45	09.48 ± 1.71	04.52 ± 1.08	08.58 ± 1.26
0.4	16.38 ± 1.70	47.48 ± 2.01	16.50 ± 1.51	07.18 ± 0.64	00.80 ± 0.34	11.50 ± 3.17
0.6	00.00	00.00	00.00	00.00	00.00	00.00
0.8	00.00	00.00	00.00	00.00	00.00	00.00

Table 29: Differential Haemocyte Counts determined in adult female following the topical application of various concentrations of Muristerone on 1-2 day old 5th instar nymphs of *Dysdercus cingulatus*

Concentrations (%) 1 µl / nymph	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Adipohaemocytes % ± S. E.	Granulocytes % ± S. E.	Oenocytoids % ± S. E.	Disintegrated Cells % ± S. E.
Untreated	05.58 ± 0.51	50.20 ± 3.64	28.06 ± 2.15	07.42 ± 1.47	01.92 ± 0.25	06.72 ± 1.46
Solvent Treated	04.66 ± 1.21	48.16 ± 4.13	23.20± 2.43	12.64 ± 1.61	04.02 ± 0.53	06.92 ± 0.61
0.2	14.20 ± 1.37	47.66 ± 5.28	13.12± 2.51	08.34 ± 1.38	04.04 ± 0.85	12.80 ± 2.33
0.4	17.28 ± 1.02	45.16 ± 2.14	12.80 ± 1.20	06.22 ± 0.45	02.00 ± 0.34	16.58 ± 2.20
0.6	00.00	00.00	00.00	00.00	00.00	00.00
0.8	00.00	00.00	00.00	00.00	00.00	00.00

Table 30: Differential Haemocyte Counts determined after 6 hrs. following the topical application of various concentrations of Methoprene on 1-2 day old 5th instar nymphs of *Dysdercus cingulatus*

Concentrations (%) 1 µl / nymph	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Adipohaemocytes % ± S. E.	Granulocytes % ± S. E.	Oenocytoids % ± S. E.	Disintegrated Cells % ± S. E.
Untreated	09.22 ± 1.23	55.60 ± 3.13	17.64 ± 2.33	12.02 ± 1.03	02.20 ± 0.29	03.44 ± 1.02
Solvent Treated	12.14 ± 0.99	55.40 ± 4.32	15.28± 2.26	09.70 ± 2.28	03.28 ± 0.47	04.28 ± 1.41
0.04	15.84 ± 1.24	58.76 ± 1.64	10.12 ± 1.48	06.28 ± 1.09	02.26 ± 0.50	06.74 ± 1.21
0.08	10.94 ± 0.85	62.28 ± 3.28	05.66 ± 1.07	06.02 ± 0.47	02.20 ± 0.31	13.04 ± 2.45
0.1	14.02 ± 1.63	51.80 ± 4.59	04.84 ± 0.78	07.08 ± 0.86	03.82 ± 0.85	18.34 ± 2.52
0.2	19.12 ± 1.80	49.54 ± 4.69	02.42 ± 0.49	03.94 ± 0.68	03.98 ± 0.39	19.94 ± 0.68

Table 31: Differential Haemocyte Counts determined after 1 day following the topical application of various concentrations of Methoprene on 1-2 day old 5th instar nymphs of *Dysdercus cingulatus*

Concentrations (%) 1 µl / nymph	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Adipohaemocytes % ± S. E.	Granulocytes % ± S. E.	Oenocytoids % ± S. E.	Disintegrated Cells % ± S. E.
Untreated	14.30 ± 1.21	51.16 ± 3.26	19.10 ± 1.26	09.24 ± 1.26	02.66 ± 0.62	03.66 ± 0.97
Solvent Treated	12.36 ± 1.29	48.84 ± 2.53	20.22± 1.75	11.64 ± 0.96	01.12 ± 0.43	05.78 ± 1.48
0.04	16.58 ± 1.66	54.16 ± 2.49	11.46 ± 0.78	06.32 ± 0.73	02.26 ± 0.32	09.30 ± 2.52
0.08	19.54 ± 2.14	53.48 ± 3.02	03.28 ± 0.74	03.56 ± 0.97	02.34 ± 0.38	17.78 ± 3.64
0.1	18.84 ± 1.80	48.92 ± 2.44	05.06 ± 0.70	01.12 ± 0.50	03.88 ± 0.94	22.20 ± 1.72
0.2	19.48 ± 2.76	45.60 ± 2.55	01.06 ± 0.47	01.10 ± 0.48	04.58 ± 0.63	28.18 ± 1.68

Table 32: Differential Haemocyte Counts determined after 3 day following the topical application of various concentrations of Methoprene on 1-2 day old 5th instar nymphs of *Dysdercus cingulatus*

Concentrations (%) 1 µl / nymph	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Adipohaemocytes % ± S. E.	Granulocytes % ± S. E.	Oenocytoids % ± S. E.	Disintegrated Cells % ± S. E.
Untreated	14.94 ± 1.32	37.76 ± 3.71	30.06 ± 3.22	09.22 ± 1.04	02.84 ± 0.51	05.18 ± 0.99
Solvent Treated	14.58 ± 1.66	36.02 ± 3.75	30.32± 33.84	12.70 ± 2.05	02.58 ± 0.65	03.80 ± 0.83
0.04	15.06 ± 2.24	34.28 ± 2.69	26.76± 1.89	08.66 ± 1.50	04.02 ± 1.59	11.24 ± 0.83
0.08	20.26 ± 1.86	40.52 ± 4.74	15.08 ± 2.57	04.94 ± 1.44	03.96 ± 0.95	15.24 ± 0.92
0.1	21.94 ± 2.26	41.96 ± 1.37	10.32 ± 1.09	03.50 ± 0.86	02.26 ± 0.83	20.06 ± 2.16
0.2	20.22 ± 2.54	45.90 ± 5.99	05.32 ± 0.43	01.58 ± 0.47	04.22 ± 0.91	22.76 ± 3.80

Table 33: Differential Haemocyte Counts determined after 5 days following the topical application of various concentrations of Methoprene on 1-2 day old 5th instar nymphs of *Dysdercus cingulatus*.

Concentrations (%) 1 µl / nymph	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Adipohaemocytes % ± S. E.	Granulocytes % ± S. E.	Oenocytoids % ± S. E.	Disintegrated Cells % ± S. E.
Untreated	06.40 ± 0.87	38.84 ± 3.05	36.68 ± 3.82	10.12 ± 1.30	03.78 ± 0.66	05.88 ± 0.63
Solvent Treated	04.36 ± 1.58	39.74 ± 2.44	34.32± 3.68	12.02 ± 2.02	03.60 ± 0.49	05.90 ± 1.08
0.04	08.98 ± 1.03	43.94 ± 2.51	29.64± 2.06	04.16 ± 0.72	03.26 ± 0.61	10.00 ± 1.09
0.08	11.30 ± 1.13	41.16 ± 1.77	25.96 ± 2.46	04.16 ± 0.54	03.96 ± 0.79	13.46 ± 1.07
0.1	16.10 ± 1.71	44.50 ± 1.88	14.82 ± 1.93	05.30 ± 0.33	05.30 ± 0.72	13.92 ± 0.73
0.2	15.18 ± 1.39	46.72 ± 5.78	11.12 ± 2.36	02.92 ± 0.60	04.88 ± 0.38	19.30 ± 2.45

Table 34: Differential Haemocyte Counts determined in adult male following the topical application of various concentrations of Methoprene on 1-2 day old 5th instar nymphs of *Dysdercus cingulatus*

Concentrations (%) 1 µl / nymph	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Adipohaemocytes % ± S. E.	Granulocytes % ± S. E.	Oenocytoids % ± S. E.	Disintegrated Cells % ± S. E.
Untreated	01.86 ± 0.57	40.38 ± 3.78	45.02 ± 4.31	04.02 ± 0.65	02.30 ± 0.34	06.42 ± 0.92
Solvent Treated	03.18 ± 0.46	36.72 ± 2.10	46.58± 2.24	05.06 ± 0.54	03.26 ± 0.66	05.00 ± 0.80
0.04	11.90 ± 1.02	41.70 ± 1.76	24.66± 1.40	07.20 ± 1.12	03.42 ± 0.51	11.08 ± 0.99
0.08	12.08 ± 1.01	45.62 ± 3.54	18.50 ± 2.02	08.26 ± 1.17	02.86 ± 0.41	12.68 ± 1.36
0.1	00.00	00.00	00.00	00.00	00.00	00.00
0.2	00.00	00.00	00.00	00.00	00.00	00.00

Table 35: Differential Haemocyte Counts determined in adult female following the topical application of various concentrations of Methoprene on 1-2 day old 5th instar nymphs of *Dysdercus cingulatus*

Concentrations (%) 1 µl / nymph	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Adipohaemocytes % ± S. E.	Granulocytes % ± S. E.	Oenocytoids % ± S. E.	Disintegrated Cells % ± S. E.
Untreated	05.20 ± 0.45	50.82 ± 6.49	26.56 ± 5.02	12.04 ± 1.42	02.88 ± 0.54	02.50 ± 0.63
Solvent Treated	07.00 ± 0.82	46.50 ± 2.30	29.90± 3.33	11.20 ± 1.15	02.36 ± 0.65	03.24 ± 0.83
0.04	12.24 ± 0.89	51.10 ± 3.34	15.32± 2.17	06.86 ± 0.84	02.32 ± 0.95	11.46 ± 1.43
0.08	13.23 ± 1.19	51.94 ± 1.39	13.88 ± 1.63	04.28 ± 0.64	03.98 ± 0.39	12.70 ± 0.86
0.1	00.00	00.00	00.00	00.00	00.00	00.00
0.2	00.00	00.00	00.00	00.00	00.00	00.00

SECTION - 2

I. Observations on free haemocytes of normal *Diacrisia obliqua*

The haemocytes of 6th instar larvae and pupae of *Diacrisia obliqua* were identified and studied under Light as well as Transmission Electron Microscope. It was found that the types of free haemocytes were same throughout 6th larval instar, although their comparative ratio differed in different stages of development.

1. Classification and description of free haemocytes of *Diacrisia obliqua*

The haemocytes of *D. obliqua* were characterized into six types after the examination of at least 5-10 smears from insects of 6th instar larval stage as well as of 1-2 day old pupae under the light microscope. The larval haemocytes were identified as prohaemocytes, plasmatocytes, granulocytes, spherulocytes, oenocytoids and coagulocytes whereas those of pupae were classified into prohaemocytes, plasmatocytes, granulocytes and coagulocytes. The characteristics of each type are as follows.

Prohaemocytes

These were small, generally round, sometimes ovoid, pear shaped or rarely irregular cells (Plate IX, Fig. A to I). The nucleus was large, sometimes ovoid, or occasionally notched, occupying most of the cell, leaving only scanty cytoplasm at the rim of the cell. Nucleus was eosinophilic containing distinct chromatin granules whereas the cytoplasm was basophillic and homogeneous, sometimes completely

obscure. The diameter of round cells varied from 3.0 μ to 7.0 μ. The ovoid cells varied between 4.6 μ to 6.5 μ in width and 5.8μ to 9.0 μ in length.

Plasmatocytes

These were highly polymorphic cells exhibiting round, oval, pear, spindle, sickle and irregular forms (Plate IX, Fig. A to I). The most common forms being oval and spindle. These cells contained larger amount of cytoplasm compared to prohaemocytes. The cytoplasm was basophillic and sometimes contains vacuoles or granules. The nucleus was round, ovoid or elongated. In the pupal stage the plasmatocytes were mostly sickle shaped. Binucleate forms were occasionally encountered. Mitotic divisions were usually observed (Plate IX, Fig. G & I). The diameter of spherical cells varies from 7.8 μ to 15.2 μ and the ovoid cells ranged from 9.0 μ to 16.2 μ in length and 9.6 μ to 13.4 μ in width. The spindle shaped cells ranged from 15.2 μ to 40.2 μ in length and 3.8 μ to 7.2 μ in width.

Oenocytoids

These were generally large, thick, round or ovoid cells, rarely pear shaped and irregular (Plate IX, Fig. H). Relatively abundant cytoplasm and small eccentric nucleus characterized the cells. The cytoplasm was usually homogeneous and was deeply basophilic, often with one or two small vacuoles. The diameter of spherical cells ranged from 12.6 μ to 18.2 μ. The ovoid cells measured 10.2 μ to 19.8 μ in length and 9.6 μ to 17.6 μ in width.

Spherulocytes

These cells were from medium to large, mostly spherical or oval, filled with many spherical spherules or globules of varying diameter (Plate IX, Fig. A to I). The cells were 7.8 to 20.2 μ long and 5.0 to 8.2 μ wide. The nucleus was small, central or eccentric in position, generally eclipsed by the presence of abundant spherules in the cytoplasm.

Coagulocytes

These cells were large, spherical, ovoid, elongated or irregular (Plate IX, Fig. A to I). The cell membrane was indistinct and the cytoplasm was comparatively achromophilic, sometimes containing long glassy crystals. Some cells appeared expanded and there were only cytoplasmic impressions around the nucleus. The nucleus was small, compact, central or slightly eccentric in position. The round cells measured about 18.2 μ in diameter. The size of ovoid cells varied between 12.6 to 19.2 μ in length and 8.6 to 14.8 μ in width.

2. Haemocytes of *Diacrisia obliqua* under Transmission Electron Microscope (TEM).

By Transmission Electron Microscopy (TEM), the haemocytes of 6th instar larvae (1-2 day old) of *D. obliqua* were characterized into five types viz. prohaemocytes, plasmatocytes, granulocytes, spherulocytes and oenocytoids. Categorization of haemocyte types under TEM is primarily based on the size and shape of the cell, nuclear cytoplasmic ratio, presence or absence or abundance of cytoplasmic granules etc. However, it is not always possible to classify all the cells into a certain type unambiguously because the criteria employed for the above do not always satisfy with cell characteristics. Therefore many intermediate cell types viz., prohaemocytes-plasmatocyte, plasmatocyte-granulocyte, granulocyte-spherulocyte forms were encountered in present investigation. Coagulocytes could not be identified under TEM which were, however, clearly distinguishable in light microscopic observations.

Prohaemocytes

Prohaemocytes were round, oval or pear shaped cells (Plate- XV, Fig. A,B & D) and some times irregular (Plate-XV, Fig. C) with large nucleus and scanty cytoplasm. In most of the cells, the cell surface seemed to be smooth without any outward projections or growth. However, in some instances several long tentacles like

outgrowths were observed on cell surface. In some other cells several small pseudopodia like protrusions were present which gave these cells an irregular appearance. The prohaemocytes were characterized by a low concentration of intracellular organelles viz., endoplasmic reticulum, Golgi complex mitochondria and other inclusions but ribosomes were numerous and free, filling the entire cytoplasm. The cytoplasm also contained a few dense inclusions of variable sizes in certain cells. The origination of endoplasmic reticulum from the out folding of outer plasma membrane was evident in some cells. The nucleus was enveloped by double membrane. The nuclei of different prohaemocytes showed variation in form such as round, oval and irregular. The chromatin material in the nuclei of a few prohaemocytes was observed to be incompletely separated. However, in most of the cells it was clearly distinguishable into heterochromatin and euchromatin. The nucleoli were usually eccentrically placed, compact and mostly composed of electron dense material. But sometimes the nucleolus was observed to be formed in such a way that an electron lucent area was left within the electron dense area.

Plasmatocytes

Plasmatocytes generally occurred in a variety of forms such as round, oval, and pear and spindle shaped or even irregular. The cytoplasmic matrix appeared dense with many well-developed organelles. The mitochondria were numerous and well developed and occurred in various shapes such as round, oval and rod shaped (Plate-XVII, Fig. G). Small profiles of Golgi complexes were occasionally seen. One plasmatocyte showed many well-developed Golgi complexes. Secretory vesicles were numerous, however, lamellae were few but large in size (Plate-XVI, Fig. D). Free ribosomes were abundantly present in the cytoplasmic matrix (Plate-XVII, Fig. C & F). The rough endoplasmic reticulum was well developed and formed large networks in the cytoplasm (Plate-XVII, Fig. A & B). In some plasmatocytes, the endoplasmic reticulum cisternae were distended and filled with fibrous substance (Plate-XVII, Fig. B). Membrane bound lysosomes which contained undigested material (residual bodies) were frequently seen (Plate-XVI, Fig. A; Plate-XVII, Fig. B). Small vacuoles were also occasionally present (Plate-XVI, Fig. B & D; Plate-XVII, Fig. A, D & E). The nucleus of plasmatocytes occupied smaller space of cytoplasm and mostly centrally

placed in the cell compared to prohaemocytes. However, the nuclei of the plasmatocytes also showed a variety of forms such as round, oval, elongated and irregular. Certain plasmatocytes (Plate-XVI, Fig. D) had small and round structure composed of nuclear material. The oval to pear shaped cells often had an oval or lobed nucleus (Plate-XVI, Fig. D & E) while spindle shaped and elongated cells are characterized by spindle shaped and elongated nucleus (Plate-XVI, Fig. B & C; Plate-XVII, Fig. E; Plate XVIII, Fig. A).

The chromatin material in the nuclei of plasmatocytes was easily distinguished into heterochromatin and euchromatin. A large number of these cells were observed to possess small tentacles like outgrowths on the surface (Plate-XVII, Fig. A) but plasmatocytes without tentacular outgrowth were also not uncommon.

Granulocytes

The granulocytes of fully-grown larvae of *D. obliqua* were recognized due to the universal presence of numerous granules in their cytoplasm. These cells were more or less spherical in shape with somewhat irregular outline. The cytoplasmic granules varied in number and distribution (Plate-XVIII, Fig. B, C & D). The granules were further classified on the basis of their electron density (Plate-XVIII, Fig. B). However, granules of high density were darker in colour and more abundant than the light colour granules. The granulocytes were further characterized with well developed smooth as well as rough endoplasmic reticulum but the former type was often in the form of distended sacs filled with fine fibrous material in the cisternal spaces (Plate-XIX, Fig. M). There were usually several Golgi complexes in a section and in the vicinity of each complex a few packed as well as clear vesicles were seen. Various granules in the developing stages are present in the close association of the Golgi complex and the endoplasmic reticulum (Plate-XIX, Fig. M).

The granulocytes often contained large deposits of glycogen like granules that were localized into two or more groups. Pinocytotic vesicles in close association with the cell membrane were occasionally seen in these cells (Plate-XIX, Fig. N). One of the regular and constant features of these haemocytes was the presence of

lysosomes which were membrane bound and often contained undigested material in the form of residual and myelinated bodies (Plate-XVIII, Fig. B; Plate-XIX, Fig. M & O). In some granulocytes the cell surface was extended into a few pseudopodia like extensions, and vacuoles were formed by coalescence of two undulating folds of these extensions (Plate-XVIII, Fig. C; Plate-XIX, Fig. P).

The granulocytes usually contained well-developed, centrally located and generally irregular or lobed nucleus having distinct heterochromatin and euchromatin regions and well-developed nucleoli. Sometimes nucleus became indistinct due to the presence of large granules in the cytoplasm (Plate-XIX, Fig. P).

Oenocytoids

There were a few oenocytoids as compared to other types of haemocytes in the haemolymph of *D. obliqua*. These were large, spherical or oval cells having characteristically eccentric nucleus (Plate-XX, Fig. M & N). The cytoplasm was homogeneous and devoid of any distinguishing cell organelles or inclusions, but one or two vacuoles and dark granules might be present. Furthermore, the cytoplasm appeared to be filled with ribosomes and fibers with microtubules. However, both, the microtubules and the ribosomes were interspersed in such a manner so as to give a hon ogeneous appearance to the cytoplasm (Plate-XX, Fig. N & O). Other organelles such as mitochondria, small granules and vesicles were usually located near the peripheral region of the cell (Plate-XX, Fig. M & N). The nucleus was small, bounded by double membrane and had well-developed and distinct heterochromatin and euchromatin. In some cells the nucleolus was large, centrally placed, compact and mostly composed of electron dense materials (Plate-XX, Fig. O). However, in others the nucleolus was not distinct and heterochromatin was arranged at the periphery of nucleus in separate patches (Plate-XX, Fig. N). A constant and interesting feature of the oenocytoids was the presence of small spheres all round the nucleus on the nuclear membrane (Plate-XX, Fig. O & P)

Spherulocytes

These were usually round, oval, rod shaped or elongated and irregular cells. They were characterized by large electron dense spherules of various sizes in the cytoplasm. The nucleus was small, some times eccentric and was usually masked by a large number of spherules. Golgi complex, mitochondria and rough endoplasmic reticulum were abundant in the haemocytes that contain few spherules. These cells were relatively very few in the section (Plate-XXI, Fig. A to D).

Cells that have structural features, characteristics of two different cell types had also been observed. An intermediate between a plasmatocyte and a granulocyte was often encountered. These intermediate cells were generally spherical (Plate-XXI, Fig. E), oval (Plate-XXI, Fig. F) and some times with irregular outline consisting of many loss ribosomes, well developed endoplasmic reticulum with distended cisternal spaces, well developed mitochondria, a few dense lysosomes like bodies and the occasional granules, characteristic of the granulocytes. Another type had cells, which are plasmatocyte-like having dense and tubular matrix with very few organelles.

3. Total Haemocyte Counts (THCs) of free haemocytes of normal *Diacrisia obliqua.*

Total haemocyte counts in untreated larvae were determined for 1, 2, 4 and 6 days old 6^{th} instar larvae as well as in 1-2 day old pupae. In one day old larvae THC varied between 17100-52150 cells/mm^3 of haemolymph with a mean value of 34716 ± 3622.24 cells/mm^3. Two days old larvae had an average of 41750 ± 5742.83 cells in one mm^3 of blood (range: 23450-65100 cells/mm^3). In 4 days old larvae mean THC was recorded as 70438 ± 7203.43 cells/mm^3 showing minimum of 43050 cells and maximum of 98350 cells/mm^3 of haemolymph. In 6 days old larvae, on an average 58934 ± 4749.40 cells were found in one mm^3 of haemolymph (range: 43350-91050 cells/mm^3). After six days, moulting started in the larvae. Furthermore, the total counts of haemocytes were recorded in 1-2 days old pupae, which exhibited on an average 9464 ± 1731.99 cells/mm^3 of haemolymph (range: 4800-17650 cells/mm3). In the

newly moulted 6th instar larvae THC is relatively low, it then almost doubled after 4 days, furthermore, after 6 days there was a slight decline in total number of haemocytes/mm^3. When 6th instar larvae moulted to pupal stage, THC dipped to its lowest as compared to other stages examined in the present experiment.

4. Differential Haemocyte Counts (DHCs) of free haemocytes of *Diacrisia obliqua*

To estimate the relative proportion of different types of haemocytes DHCs were determine in the 6th instar larvae and pupae (age-wise as mentioned in case of THC). The prohaemocytes, plasmatocytes, granulocytes, oenocytoids, coagulocytes and spherulocytes were the main larval haemocytes whereas in the pupae, only prohaemocytes, plasmatocytes, granulocytes and spherulocytes were present. The relative percentage of different types of haemocytes was calculated in both larvae and pupae on the basis of their respective numbers in total number of cells counted as described in "Material and Methods".

The average population of prohaemocytes in newly moulted 6th instar larvae was 33.96%, which underwent progressive reduction as the age of 6th instar larvae increased. By 6th day the prohaemocyte population was reduced to 24.86%. Furthermore, in 1-2 day old pupae it fell further 16.51%. Plasmatocytes constituted about half of the total cell population and showed only slight variation throughout last larval instar. When 6th instar larvae transformed to pupae, the plasmatocyte population increased to 71.21%.

On the other hand granulocytes underwent a progressive increase as the age of 6th instar larvae increased. Their population in one-day-old larvae was 2.94% that increased to 12.16% in 6 day old larvae. However 2 day old pupae were found to contain 3.57% granulocytes. Spherulocytes too underwent increase as the age of larvae progressed. It increased from 1.72% (in one-day-old larvae) to 3.58% (in 6-day-old 6th instar larvae). Whereas in 2 day old pupae these cells could not be identified

Oenocytoids constituted 1.54% of total haemocyte population in one-day-old larvae. Their population increased to 2.14% by 4th day, however, just before moulting i.e. on 6th day they reduced to 1.91%. Moreover, in pupae the oenocytoids were generally absent. Similarly, coagulocytes also were poorly represented in comparison to prohaemocytes and plasmatocytes. Their population fluctuated inconsistently throughout 6th larval instar. In two-day-old pupae they constituted 1.86% of total cell population.

II. Observations on free haemocytes of *Diacrisia obliqua* treated with different chemicals under Light Microscope.

Four concentrations, ranging from Lc30-Lc90 in case of organophosphate, carbamate and pyrethroid and Lc20-Lc60 in case of juvenoid and ecdysteroid, were applied topically to 1-day-old 6th instar larvae of *Diacrisia obliqua*. The mortality, moulting and haemocyte picture were subsequently observed after 6 hrs., 1 day, 3 days, 5 days and finally in 1-2 day old pupae.

1. Effect of topical application of different concentrations of Acephate (an organophosphate) on *Diacrisia obliqua*.

When 1 to 2 day old 6th instar larvae were treated with 0.04, 0.08, 0.1 and 0.2% acephate, the respective mortalities after 24 hrs were approximately 28-30%, 45-51%, 69-72% and 82-86% in comparison to about 1-2% in control. Feeding was adversely affected initially by all the applied concentrations, however, after one or two days, the larvae affected with lower concentrations resumed feeding. When the drop of insecticide was topically applied, the larvae responded by rapid backward movements of head and coiling of the body. With respect to lower concentrations (0.04 and 0.08%), the pharmacological symptoms were less severe and short lived and the treated larvae showed complete recovery in three days. On the other hand, following 0.1 and 0.2% acephate, paralysis occurred within 5-7 hrs. of treatment. The larvae

treated with 0.1% acephate showed partial recovery in about 15% individuals and some resumed feeding after two days of application. Whereas insects could not survive beyond 5 days when treated with the highest concentration. Feeding was almost inhibited in these larvae. The treated larvae appeared shrunken in size and dehydrated which might be due to excessive discharge of fluid from mouth as well as from anus. In the larvae treated with 0.1 and 0.2% acephate the hairs present on the surface where the drop of insecticide was placed were burnt leaving the area hairless.

1.1 Haemocyte picture of 6th instar larvae and pupae of *Diacrisia obliqua* affected with different concentrations of Acephate

After 6 hrs of application of 0.04 and 0.08% acephate, the blood picture was almost normal. When the application was made with 0.1% concentration (Plate-X, Fig. A), the plasma slightly thickened in consistency. The spherulocytes showed discharge of spherules in the smear. Prohaemocytes appeared almost like those of normal. The plasmatocytes showed vacuolization in the cytoplasm, ragged cell membrane, and discharge of cytoplasm into the plasma. In some plasmatocytes small finger like projections appeared. Granulocytes were less affected than plasmatocytes. The oenocytoids appeared like those of normal. The smear was not homogeneous in appearance. In majority of places clumping of haemocytes was evident. In these coagulated areas transparent cells of variable shapes appeared. After application of the highest concentration of acephate spherulocytes showed complete disintegration. Granulocytes and majority of plasmatocytes were filled with small vacuoles in the cytoplasm as well as in nucleus. Oenocytoids showed hypertrophy of nucleus. and the cytoplasm with a few vacuoles. The plasma was viscous in consistency and developed many folds in the smear. The haemolymph contained a large number of cells in disintegrating condition. Various stages of mitotic cell division were visible even in the damaged haemocytes.

After 1 day following treatment, the blood smears (Plate-X, Fig. B) of affected 6th instar larvae contained more plasmatocytes with pathological conditions characterized by vacuolization, extension of small finger like structures from the

surface of the cells, or a single, large extended process with discharge of cytoplasm from the terminal portion, clumping of chromatin material and disintegration of nuclear apparatus etc. Granulocytes and spherulocytes were either disintegrated beyond recognition or were so filled up with vacuoles that they could not be identified unambiguously These characteristics mostly appeared in the majority of the cells affected with 0.1 and 0.2% acephate. The coagulocytes were mostly present in those areas of smear where haemocyte density was very high. The haemocytes present in such areas were smaller in size and stained in such a way that clear distinction between nucleus and cytoplasm was difficult. In the highly affected portions the coagulocytes showed discharge of cytoplasm into nucleus. The vacuoles were rarely encountered in disintegrating coagulocytes. The lower concentrations induced less severe pathological symptoms and most of the haemocytes appeared like those of normal (Plate-X, Fig. E).

After 3 days, following application of 0.04% acephate a few haemocytes were in state of disintegration. The prohaemocytes and oenocytoids appeared like those of normal cells Granules appeared in more haemocytes than control. Following 0.08% acephate treatment the spherulocytes had disintegration and discharge of spherules into the plasma. Plasmatocytes developed vacuolation of cytoplasm as well as of nucleus. However, the prohaemocytes and oenocytoids appeared normal. There was clumping of haemocytes sporadically. Following application of the 0.1% acephate, the granulocytes were difficult to distinguish because of appearance of vacuoles in their cytoplasm. The plasmatocytes were most severely affected type of haemocytes. The cell membrane was irregular and ruptured at many places, accompanied with discharge of cell contents. The nuclear envelope was ruptured at many places and in most of the plasmatocytes, clumping of chromatin material was present. Prohaemocytes did not show much damage as compared to plasmatocytes (Plate-X, Fig. C & D).

After 5 days following treatment with the highest concentration (0.2%), the haemolymph became highly viscous. There was clumping of a large number of haemocytes. The haemocyte population was very dense in many areas in the smear, which had very darkly stained haemocytes, consequently the extent of damage in

these haemocytes was difficult to assess. However, there were many areas in the smear that exhibited a homogeneous distribution and proper staining of haemocytes. The plasmatocytes had severe histopathological abnormalities (Plate-X, Fig. G & H). Prohaemocytes, too, had irregular cell membrane and distortion of cell shape. The nucleus contained many vacuoles, in some cells the nucleus was broken into pieces. In prohaemocytes, most of the histopathological abnormalities appeared in the nucleus rather than in cytoplasm. Some oenocytoids had irregular cell membrane, enlarged nucleus, which sometimes showed clumping of chromatin material. Some mitotically dividing haemocytes exhibited severe histopathological conditions. Although majority of the haemocytes in the smear showed histopathological abnormalities, a fair population of haemocytes appeared quite normal. The predominant haemocyte type in such population was prohaemocyte which were supposedly freshly released into the haemolymph from haemopoetic organs or became free from the surface of different organs on which they normally rest, to replenish the dwindling cell population.

On the other hand, in case of application of lower concentrations viz. 0.04 and 0.08% acephate, the majority of haemocytes appeared normal. Even the affected larvae showed an overall recovery from the effect of insecticide.

In the successfully transformed pupae, aged 1-2 days, the haemocyte picture was like that of normal pupae. The smear contained very few cells with histopathological abnormalities (Plate-X, Fig. F). However, in some of the affected pupae the prohaemocytes population was slightly more than that of the control.

1.2 THCs of 6th instar larvae and pupae of *Diacrisia obliqua* affected with different concentrations of Acephate

THCs of larvae recorded after 6 hrs. 1 day, 3 days and 5 days following treatment with each of the selected concentration of acephate are summarized in table-36. When the treated 6th instar larvae moulted to pupal stage, the total counts were recorded in one-day-old pupae. The THCs at different time intervals were as follows.

After 6 hrs

The THCs of untreated and acetone treated larvae (control) of respective age and stages were recorded as 35590 ± 2504.92 and 34310 ± 2998.97 haemocytes/mm^3 of blood, respectively. Following treatment with 0.04 and 0.08% acephate, the counts increased by 21.77 and 33.26% as compared to control. Whereas after the treatment with 0.1 and 0.2%, THCs were reduced by 20.26% ($t= 1.2071$, $P>0.05$) and 57.07 % ($t= 2.8487$, $P<0.05$), respectively exhibiting significant reduction at the highest concentration of acephate after 6 hrs.

After 1 day

The THCs were increased by 25.83 and 18.56% following the application of 0.04 and 0.08% acephate, respectively. On the other hand, treatment with the higher concentration viz. 0.1 and 0.2% caused a reduction of 31.80 and 77.18% in the total counts of haemocytes compared to the control. The highest concentration induced a statistically significant reduction ($t= 3.5498$. $P<0.05$)

After 3 days

The THCs of untreated and solvent treated (control) 6^{th} instar larvae of the corresponding age were 72830 ± 3896.17 and 73000 ± 4752.39 cells/mm^3 respectively. All the selected concentrations of acephate reduced the total cell counts. The highest concentration caused a statistically significant reduction in THC as compared to control ($t= 3.3360$, $P<0.05$).

After 5 days

The normal and acetone treated larvae (control) at this stage exhibited total cell counts as 60410 ± 3822.15 and 63800 ± 6787.07 per mm^3 of haemolymph. The lowest concentration (0.04%) slightly increased (6.60%) the THC. Whereas other three higher concentrations viz. 0.08, 0.1 and 0.2% reduced the THCs by 17.08, 40.17 and

64.33%, respectively. In spite of a high reduction at stronger concentrations the results were insignificant at 5% level due to great variation in the counts of individuals of the same batch.

After pupal ecdysis

The changes appearing in THCs of 1 day old pupae affected with various concentrations of acephate were insignificant as compared to control.

1.3 DHCs of 6th instar larvae and pupae of *Diacrisia obliqua* affected with different concentrations of Acephate.

In addition to THC, DHCs of treated 6th instar larvae were determined after 6 hrs, 1 day, 3 days and 5 days following the application. The DHC of 1-day-old pupae was also calculated after the treatment with various concentrations. The DHCs at different time intervals were as follows.

After 6 hrs (Table- 41, Fig. 20)

Following treatment with 0.04, 0.08, 0.1 and 0.2% acephate, prohaemocytes were found to be 2.02, 9.98 3.04 and 7.94% more compared to control which exhibited 38.36% prohaemocytes thus showing an inconsistent and statistically insignificant increase in their population. Plasmatocytes were slightly enhanced by the lowest concentration, however, other serial concentrations of acephate induced a dose based reduction. Granulocytes showed inconsistent variation in their relative population in comparison to control. Spherulocytes were poorly represented in the blood smears and were completely absent by the highest concentration (0.2%). Oenocytoids were significantly increased at higher concentrations of acephate. Coagulocytes showed linear increase in their population following the treatment with increasing concentrations of acephate ($Y= 0.64 + 21.90 X$, $r = 0.9794$, $P< 0.001$). Unclassified and damaged cells constituted 10.36% of total cell population in the smear, respectively, increasing to 11.26, 12.48, 16.00 and 28.16% following the treatment

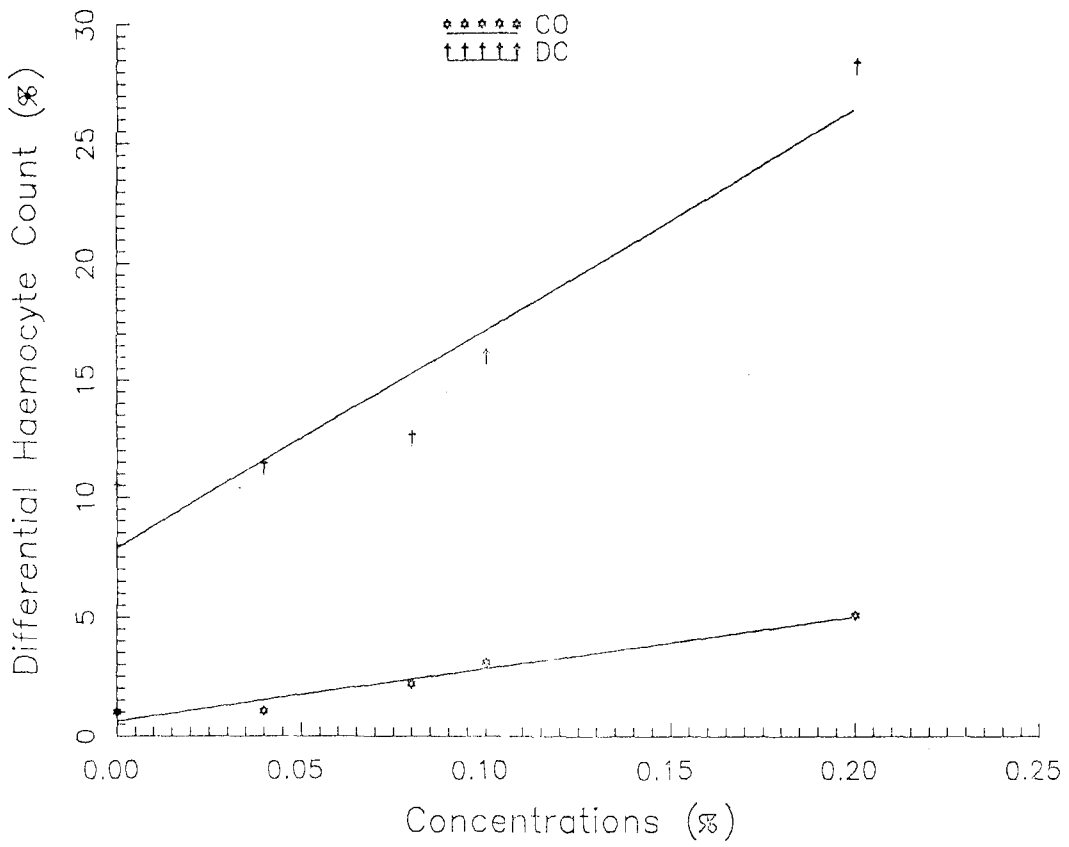

Fig. 20: Correlation between the Differential Haemocyte Count (%) and various concentrations of acephate after 6 hrs following the treatment on one day old 6th instar larvae of *Diacrisia obliqua*

with 0.04, 0.08, 0.1 and 0.2% acephate thus showing a positive linear correlation (Y= 7.86 + 92.74 X, r = 0.9557, P < 0.001).

After 1 day (Table-42, Fig.21)

After one day following the treatment with 0.04 and 0.08% acephate, there was slight increase in prohaemocyte population, however, application of the higher concentrations resulted in moderate fall in these cells as compared to control. Plasmatocytes exhibited a negative linear correlation (Y = 42.94 - 116.08 X, r = - 0.8993, P<0.001) with increase in acephate concentration. Granulocytes showed inconsistent variation in their percentage in the affected larvae with various concentration of acephate. Spherulocytes constituted 3.06% of total population in the control, which showed a reduction in affected larvae. On the other hand oenocytoids were increased with respect to increasing concentrations of acephate. Coagulocytes although showed a reduction at 0.08% acephate, they generally exhibited a tendency for enhancement in the affected larvae. The disintegrating cells were significantly increased thus showing a positive linear correlation with increase in acephate concentration (Y= 6.28 + 184.52 X, r = 0.9282, P<0.001)

After 3 days (Table-43, Fig. 22)

Prohaemocytes, after 3 days of application of various concentrations of acephate indicated an inconsistent increase in their population whereas plasmatocytes showed a concentration based linear reduction (Y= 53.58 - 207.72 X, r = - 0.9275, P<0.001) which was statistically significant at two higher concentrations (t=3.159 and t=4.285, P<0.05). Granulocytes could not be identified following the treatment with higher concentrations. Similarly, spherulocytes, too, were absent in the smears of insects treated with 0.08, 0.1 and 0.2% acephate whereas oenocytoids percentage was linearly increased with increasing concentrations (Y = 0.27 + 69.50 X, r = 0.9726, P<0.001). Similarly coagulocytes also yielded positive linear correlation affected with the increasing concentrations (Y = 1.55 + 23.67 X, r = 0.9165, P<0.001). The population of disintegrating cells was consistently increased thereby showing a

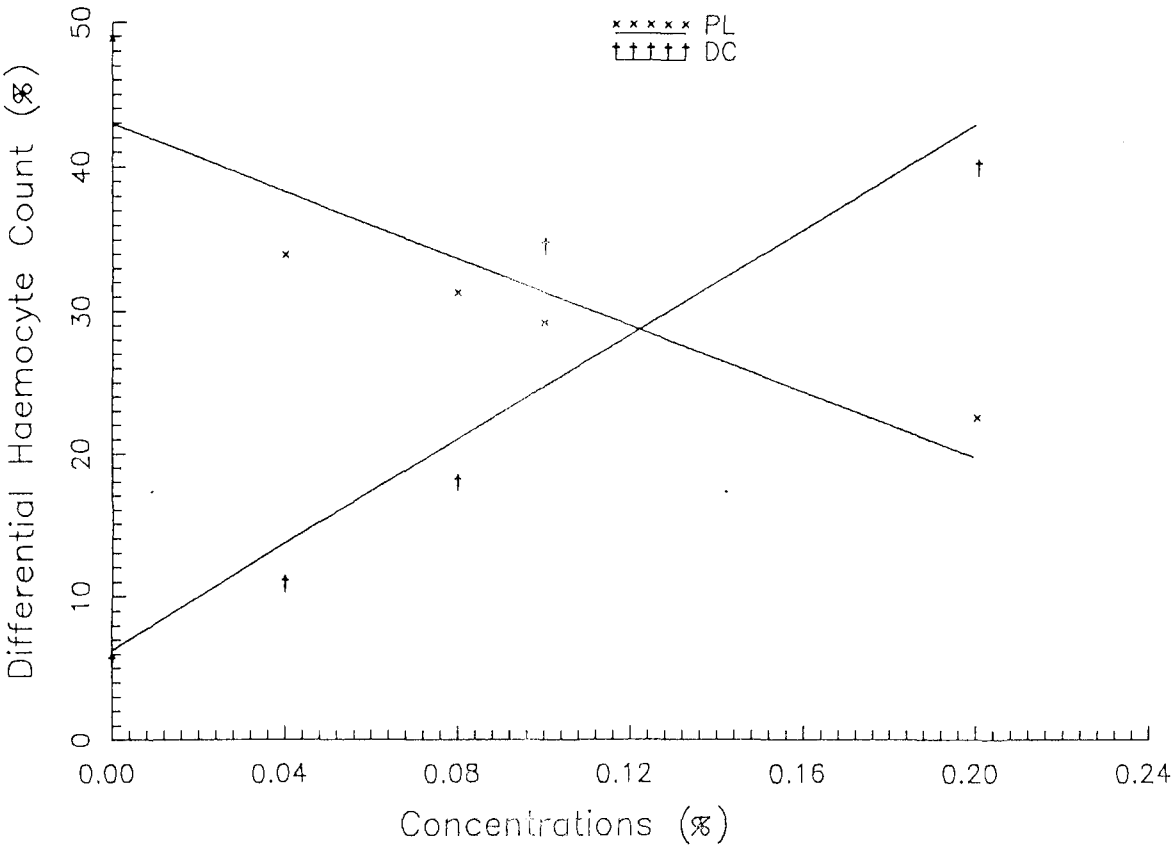

Fig. 21: Correlation between the Differential Haemocyte Count (%) and various concentrations of acephate after 1 day of the treatment on one day old 6th instar larvae of Diacrisia obliqua.

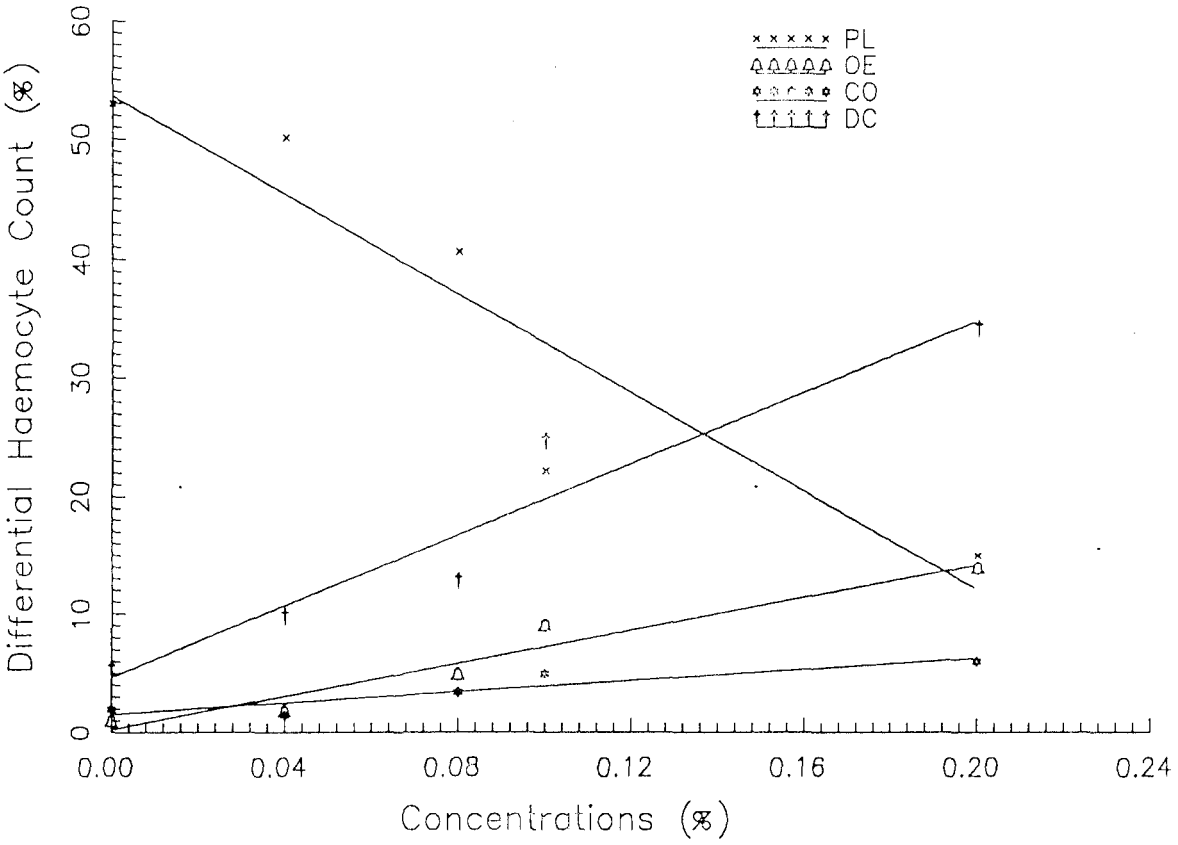

Fig. 22 : Correlation between the Differential Haemocyte Count (%) and various concentrations of acephate after 3 days of the treatment on one day old 6th instar larvae of Dicranisia obliqua.

positive linear correlation with increasing concentrations of acephate (Y = 4.68 + 150.70 X, r = 0.9627, P<0.001).

After 5 days (Table-44, Fig. 23)

Prohaemocyte number was slightly enhanced when affected with different concentrations after 5 days of application. Plasmatocytes showed a consistent reduction in population with increase in the acephate concentration. The regression between plasmatocyte population and acephate concentration showed negative linear correlation (Y= 43.72 - 154.67 X, r = -0.9918, P<0.001). Granulocytes were slightly (and insignificantly) increased with lowest concentration. Other concentrations had a negative effect on granulocytes leading to complete destruction by the highest concentration. Oenocytoids population was slightly increased. Coagulocytes percentage increased to as many as three times compared to control and exhibited a positive linear correlation with increasing concentrations (Y = 1.69 + 28.40 X, r = 0.9745, P<0.001). Disintegrating haemocytes constituted 7.06% of total cell population in the control group. These cells linearly increased in percentage with the increase in concentration of acephate (Y= 6.24 + 189.16 X, r = 0.9529, P<0.001).

After pupal ecdysis (Table-45)

The blood smears of pupae affected with 0.04, 0.08, and 0.1% acephate contained 4.56, 9.14 and 7.38% less prohaemocytes, respectively, as compared to control showing a statistically insignificant reduction. Plasmatocytes showed slight enhancement in their population, which was statistically insignificant. Spherulocytes and oenocytoids were absent in the blood smears of control as well as that of treated insects. Granulocyte population was reduced to half following application of 0.04% acephate as compared to control whereas following higher concentrations these cells could not be identified in the blood smear. Coagulocyte population did not show any appreciable alteration with two lower concentrations, however, with the 0.1% acephate these were completely absent. In case of damaged cells, the 0.1% acephate doubled their proportion as compared to control.

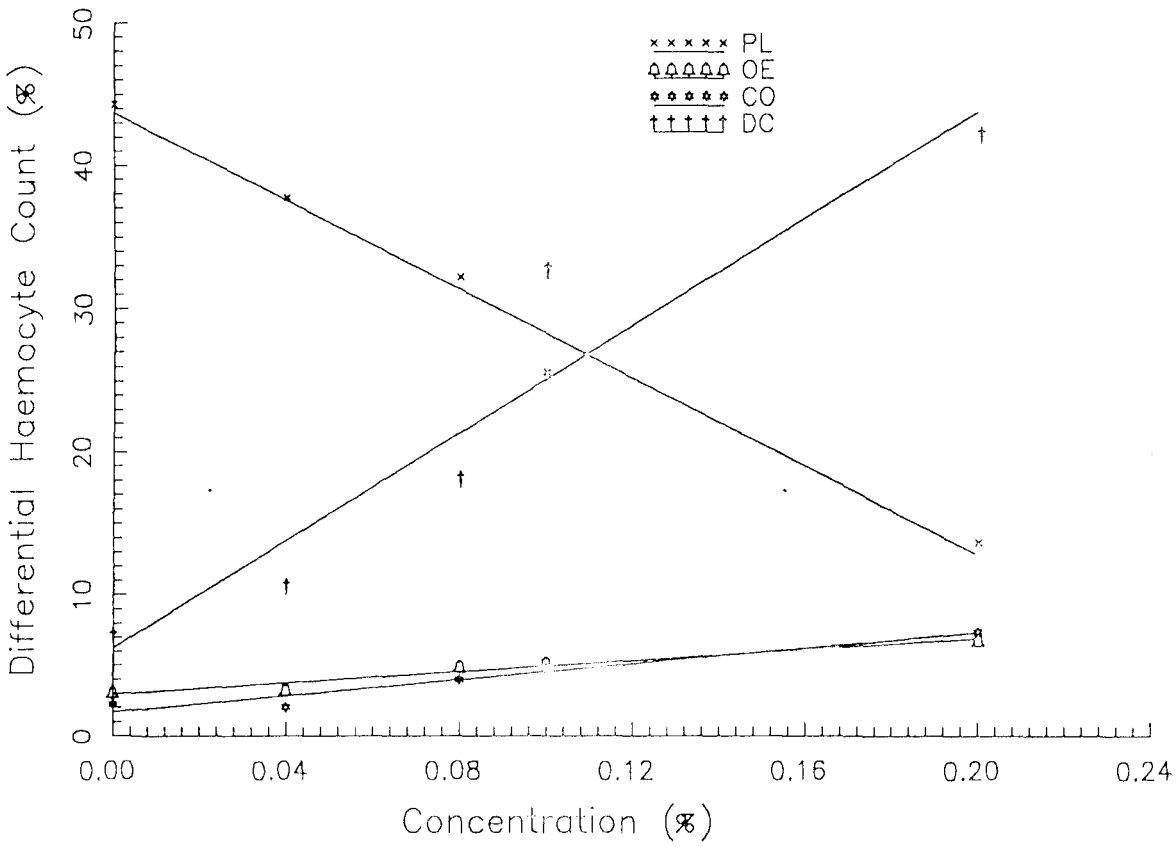

Fig. 23 : Correlation between the Differential Haemocyte Count (%) and various concentrations of acephate after 5 days of the treatment on one day old 6th instar larvae of Diacrisia obliqua.

2. Effect of topical application of different concentrations of Aminocarb (a carbamate) on *Diacrisia obliqua.*

As mentioned in case of acephate the four selected concentrations of aminocarb viz. 0.1, 0.2, 0.4 and 0.6% resulted in approximately 30-34%, 51-55%, 70-74% and 85-89% mortalities respectively up to the pupal stage. The pharmacological symptoms were concentration based. The larvae apparently recovered from pharmacological symptoms after 0.1 and 0.2% aminocarb treatment. Partial recovery was observed in case of 0.4% aminocarb. However, following the application of highest concentration all the treated larvae died before pupal ecdysis and even the prepupal stage was not attained by them. Feeding was adversely affected. The most severe symptoms included vomiting and discharge of green colour fluid from anus. The hairs were burnt at the surface where drop of insecticide was placed.

2.1 Haemocyte picture of 6th instar larvae and pupae of *Diacrisia obliqua* affected with different concentrations of Aminocarb.

After 6 hrs of application of lowest concentration of aminocarb (0.1%), the blood picture was almost like that of control. Following 0.2% aminocarb application spherulocytes contained less spherules, sometimes only 2-3 spherules were visible in cytoplasm. Vacuolation was observed in some granulocytes. Oenocytoids and coagulocytes were mostly normal. Plasmatocytes showed presence of a few small vacuoles in the cytoplasm. Treatment by 0.4% aminocarb, resulted in appearance of small vacuoles in the cytoplasm of a large number of haemocytes therefore distinction between granulocytes, spherulocytes and some plasmatocytes became difficult. Plasmatocytes exhibited vacuolization in their cytoplasm as well as in nucleus, however, majority of these haemocytes were still distinguishable (Plate-XI, Fig. B). Following the treatment with the highest concentration of aminocarb, the blood smear exhibited clumping of haemocytes at various places (Plate-XI, Fig. C). Furthermore, nearly half of the total cell population was damaged beyond recognition. In these cells

severe nuclear and cytoplasmic disintegration occurred. Since prohaemocytes and plasmatocytes constituted the majority of the cell population, and other type of haemocytes viz. spherulocytes, granulocytes, oenocytoids and coagulocytes, all four combined, constituted approximately 10% of the total haemocytes, even the drastic disintegration in the latter did not affect the total count of haemocytes much. On the other hand any drastic change in prohaemocyte or plasmatocyte population had a significant impact on the total and differential count of haemocytes. After 6 hrs following treatment with the highest concentration spherulocytes and coagulocytes were severely damaged. Granulocytes had intense vacuolization of cytoplasm and nucleus. Although a fair percentage of prohaemocytes had disintegrated nucleus, the remaining of these haemocytes appeared like those of control.

After one day following treatment with 0.1 and 0.2% acephate spherulocytes were highly affected. Coagulocytes and oenocytoids were not much affected (Plate-XI, Fig. G). There existed ambiguity in the identification of granulocytes due to vacuolization in their cytoplasm. Prohaemocytes and plasmatocytes were not severely affected. However, some plasmatocytes developed bulging of cytoplasm as well as filament like cytoplasmic processes. Some plasmatocytes also showed achromophilia. Following the application of 0.4% aminocarb, the haemocytes generally indicated the same pathological conditions as observed with respect to 0.1% acephate. Granulocytes and spherulocytes were damaged beyond recognition due to intense vacuolization of cytoplasm. Plasmatocytes had abnormal cell shape, vacuolated cytoplasm having small and large sized vacuoles, which probably represent the loss of material from the cell (Plate-XI, Fig. D), abnormal cytoplasmic extensions and in some cases out flow of cytoplasmic material. In some plasmatocytes nuclei were pushed towards the periphery. The prohaemocytes were largely unaffected, however, some severely affected cells showed disintegration of nuclear material, loss of cytoplasmic portion, distorted nuclear envelop etc. In general, most of the affected haemocytes showed the precipitation of their nuclear chromatin into large granules. This nuclear picture was comparable to the beginning of prophase of mitosis. Advanced mitotic phases were also observed which apparently took place at the same rate as in the normal haemocytes. After the application of 0.6% aminocarb, there was an apparent loss of cell number along with a decrease in the volume of haemolymph. Moreover

majority of the cells had ragged or broken membranes, enhanced cellular disintegration. There was complete destruction of granulocytes and spherulocytes. Clumping of cells was frequent. The haemocytes appeared shrunken at certain places of the smear and were darkly stained. The nuclei of oenocytoids become swollen and in many cells were pushed towards periphery and appeared as if these were about to be expelled out of the cells. The plasmatocytes showed severe histopathological abnormalities in cytoplasm as well as in nucleus (Plate-XI, Fig. F & H). The cytoplasmic changes included abnormal vacuolation, staining reactions, rupturing of cell membrane as well as nuclear membrane, discharge of cell contents, formation of bulges or tentacle like processes on the surface as well as achromophilia etc. The nuclear changes were expressed as hypertrophy, karyorrhexis and atrophy as well as occasional achromophilia.

After 3 days following treatment with lowest concentration (0.1%) of aminocarb haemocytes showed abnormal vacuolization in only a few cells otherwise as a whole the haemocyte picture appeared normal (Plate-XI, Fig. E). The treatment with next higher concentration made the spherulocytes unrecognizable. Oenocytoids and coagulocytes appeared normal. Some plasmatocytes had vacuolization in their cytoplasm and nucleus. Achromophilia and/or poor staining of haemocytes were also evident in some smears. Following 0.4% aminocarb application granulocytes, spherulocytes and majority of coagulocytes were distorted beyond recognition. Plasmatocytes showed severe cytoplasmic and nuclear irregularities as described earlier. Prohaemocytes and oenocytoids were largely unaffected or developed mild histopathological deformities (Plate-XI, Fig. A).

After 5 days with respect to lower concentrations (0.1 and 0.2%) the haemocytes appeared normal. Even spherulocytes and plasmatocytes did not show histopathological deformities. With the application of 0.4% aminocarb, the haemocytes exhibited moderate abnormalities. The spherulocytes were damaged beyond recognition. However, five days post treatment with 0.6% aminocarb, the 6^{th} instar larvae yielded very little haemolymph which was highly viscous and yellowish in colour. About half of the haemocyte population became unrecognizable due to varying degree of disintegration and damage. Still prohaemocytes and plasmatocytes were

predominant types of haemocytes (Plate-XI, Fig. I). Plasmatocytes exhibited gross cytoplasmic and nuclear abnormalities as described earlier, however, due to presence of scanty cytoplasm prohaemocytes mostly showed nuclear histopathology viz. abnormal vacuolization, nuclear fragmentation or distortion of the nuclear material and precipitation of chromatin material. In spite of severe disintegration of a large number of haemocytes, some haemocyte appeared quite normal and even comparable to the haemocytes of normal larvae.

After treatment with the highest concentration the larvae could not survive beyond five days, therefore haemocyte picture of pupae affected with the highest concentration could not be observed. Following application of other concentrations, the 1-2 day old pupae contained majority of haemocytes with very slight or no pathological abnormalities as compared to the control. However, a few haemocytes particularly plasmatocytes underwent vacuolization of cytoplasm, irregularity of cell membrane and distortion of cell shape when affected with 0.4% aminocarb. In addition to that prohaemocyte population was increased in comparison to control.

2.2 THCs of 6th instar larvae and pupae of *Diacrisia obliqua* affected with different concentrations of Aminocarb.

The THCs of larvae affected, with the 0.1, 0.2, 0.4 and 0.6% aminocarb were recorded after 6 hrs, 1 day, 3 days and 5 days following the treatment. THCs of 1-day-old pupae were also determined. The age-wise THC is summarized in table-37.

After 6 hrs

In the larvae of untreated and control stock of the respective age, the THCs were 36790 ± 3137.50 and 36860 ± 4534.93 cells/mm^3. After the treatment with the lowest concentration (0.1%) the total count was increased by 26.02% in comparison to control. On the other hand, following treatment with 0.2, 0.4 and 0.6% aminocarb the counts were reduced by 20.75%, 33.02% and 64.24% (t=1.502, t=2.049 and t=2.672 respectively, P>0.05), respectively, as compared to control.

After 1 day

The total cell counts showed 20.72% increase after 1 day following the treatment of 6th instar larvae with the lowest concentration compared to that of control larvae which contained 40830 ± 5662.23 haemocytes/mm^3. The higher concentrations resulted in progressive reduction in the cell count which was significant with the highest concentration (79.06%, $t = 2.992$. $P<0.05$).

After 3 days

Similar trend as mentioned above was observed in THCs of affected larvae after 3 days following treatment.

After 5 days

After 5 days following treatment with 0.1, 0.2, 0.4 and 0.6% aminocarb, the cell counts dropped by 8.65, 18.88, 49.85 and 68.69%, respectively, in comparison to control larvae which contained 65400 cells/mm^3 of haemolymph. The two higher concentrations (0.4 and 0.6%) induced a statistically significant reduction at 5% level ($t= 3.161$, $P<0.05$).

After pupal ecdysis

One-day-old pupae of untreated and control stock, respectively, contained 11160 ± 3096.93 and 10880 ± 1992.52 cells/mm^3 of haemolymph. The pupae affected with the lowest concentration of aminocarb showed a moderate but insignificant increase in THC. Whereas 0.2 and 0.4% aminocarb resulted in 20.50% and 33.73% reduction in the total cell count as compared to control. Moreover, with respect to the highest concentration, formation of pupae did not take place as all the treated larvae died before reaching the pupal stage.

2.3 DHCs of 6th instar larvae and pupae of *Diacrisia obliqua* affected with different concentrations of Aminocarb.

After 6 hrs (Table-46, Fig. 24)

The relative proportion of prohaemocytes 6hrs after the treatment with various concentrations varied inconsistently compared to control. Plasmatocyte population was slightly increased with the application of two lower concentrations, however, the higher concentrations viz. 0.4 and 0.6%, respectively, reduced their population to approximately half and one third of the control. Spherulocytes were 1.4 and 1.54% less than control when affected with 0.1 and 0.2% aminocarb whereas with the higher concentrations these haemocytes were absent in the blood smear. On the other hand oenocytoids were 1.08, 1.62, 3.44 and 9.28% following treatment with 0.1, 0.2, 0.4 and 0.6% aminocarb, compared with control which contained 1.32% oenocytoids. Coagulocyte population was slightly and insignificantly ($P>0.05$) enhanced after 6 hrs following application with 0.1, 0.2 and 0.4% aminocarb, however, with the highest concentration these cells could not be identified in the smears. On the other hand population of the damaged cells increased with the increase in concentration of aminocarb which was significant at 0.4 and 0.6% aminocarb ($t=4.551$ and $t=3.291$, $P<0.05$). The regression between the dose strength and percentage of damaged cells yielded a positive linear correlation ($Y= 4.19 + 54.88 X$, $r= 0.9840$, $P<0.001$).

After 1 day (Table-47, Fig. 25)

Prohaemocytes underwent statistically insignificant increase in population compared to control except at the highest concentration ($t= 2.791$, $P<0.05$). On the other hand, plasmatocytes were proportionately decreased in percentage following the application of progressively higher concentrations of aminocarb thereby showing a negative linear correlation coefficient ($Y= 51.976 - 74.893 X$, $r = - 0.9860$, $P<0.001$). The reduction was significant by 0.4% aminocarb ($t=2.786$ and $t=5.171$ respectively, $P<0.05$). Granulocytes could not be identified in the smears affected with 3 higher doses. Similarly spherulocytes, too, were absent from the smears affected with two

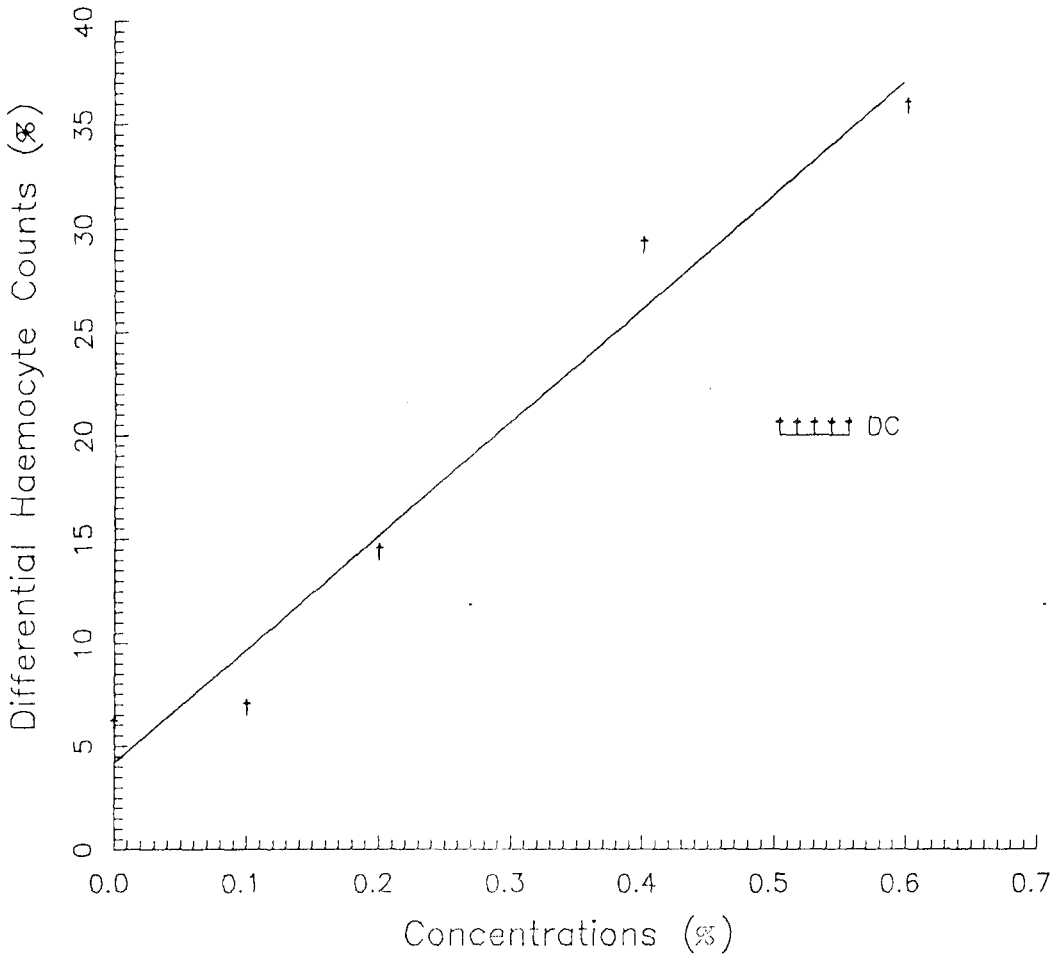

Fig. 24 : Correlation between the Differential Haemocyte Count (%) and various concentrations of aminocarb after 6 hrs of the treatment on one day old 6th instar larvae of Diacrisia obliqua.

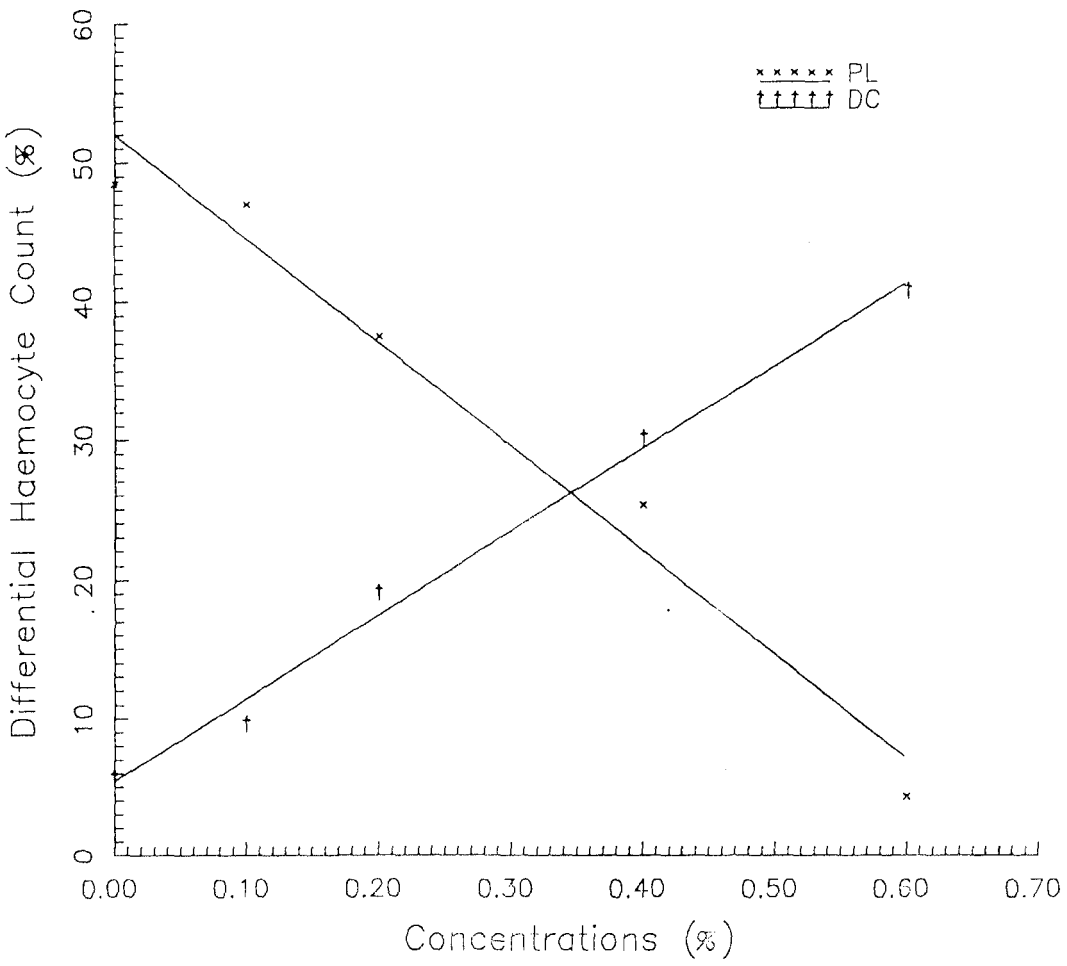

Fig. 25 : Correlation between the Differential Haemocyte Count (%) and various concentrations of aminocarb after one day of the treatment on one day old 6th instar larvae of Diacrisia obliqua.

higher concentrations. Oenocytoids were relatively less affected by the aminocarb treatment and exhibited a consistent increase in the population which was significant with the highest concentration (t= 3.015, P<0.05). Similarly the application of stronger concentrations of aminocarb resulted in positive linear correlation with the population of damaged and disintegrating cells (Y = 5.451 + 60.03 X, r = 0.9959, P<0.001). There were 9.62% (t= 1.981, P>0.05), 19.06% (t=3.096,P<0.05), 30.16% (t = 3.499, P<0.05) and 40.78% (t=4.951, P<0.05) damaged cells following application of 0.1, 0.2, 0.4 and 0.6% aminocarb, respectively, compared to 5.68% in control.

After 3 days (Table-48, Fig. 26)

After 3 days of treatment with 0.1, 0.2 and 0.4% aminocarb to 6th instar larvae the prohaemocyte population became respectively as high as 29.06% (t=1.322, P>0.05), 37.68% (t= 2.436, P>0.05) and 48.14% (t=3.916, P<0.05) compared to control in which prohaemocytes constituted 25.54% of total haemocytes. However following the application of highest concentration it fell to 32.64% (t=2.039, P>0.05). Plasmatocytes, on the other hand, exhibited a negative linear correlation with increase in concentration (Y= 55.176 - 78.616 X, r = - 0.9870, P<0.001). The reduction in plasmatocyte population was significant by 0.2, 0.4 and 0.6% aminocarb (t=2.976, t=3.478 and t=5.414, respectively, P<0.05). Granulocytes, spherulocytes, and coagulocytes were absent in the blood smear of larvae after 3 days of treatment with 0.4% and 0.6% aminocarb. Oenocytoids population was relatively increased significantly (t= 2.923, P<0.05) following the application of even the highest dose. The relative population of disintegrating cells was increased from 7.78% in control to 11.46% (t=1.942, P>0.05), 17.78% (t=2.433, P>0.05), 26.08% (t=3.178 P<0.05) and 49.62% (t=5.292, P<0.05) after the application with 0.1, 0.2, 0.4 and 0.6% aminocarb and was significant with two higher concentrations, thus exhibited a positive linear correlation (Y = 5.063 + 67.236 X, r = 0.9724, P<0.001).

After 5 days (Table-49, Fig. 27)

Prohaemocytes population showed linear increase with increasing concentrations of aminocarb (Y = 28.429 + 17.855 X, r = 0.9743, P<0.001) were

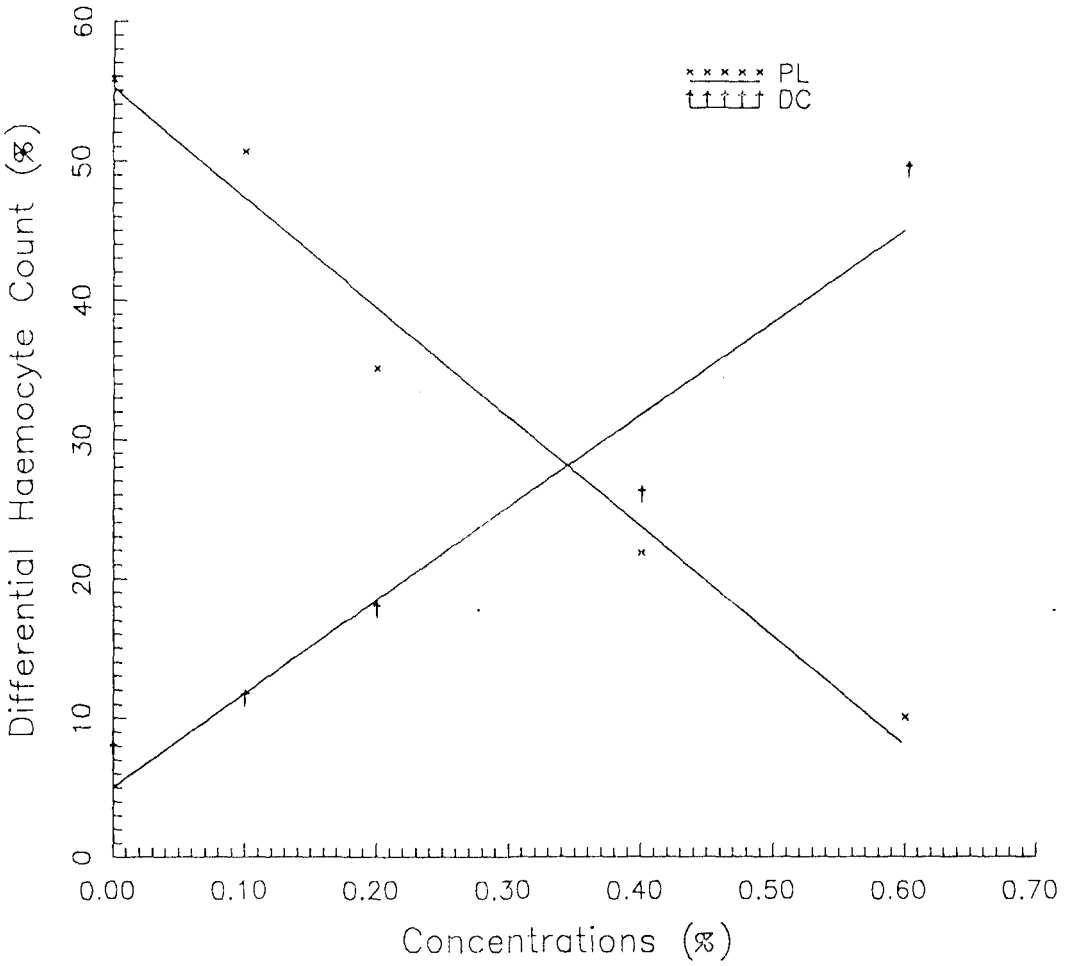

Fig. 26 : Correlation between the Differential Haemocyte Count (%) and various concentrations of aminocarb after 3 days of the treatment on one day old 6th instar larvae of Diacrisia obliqua.

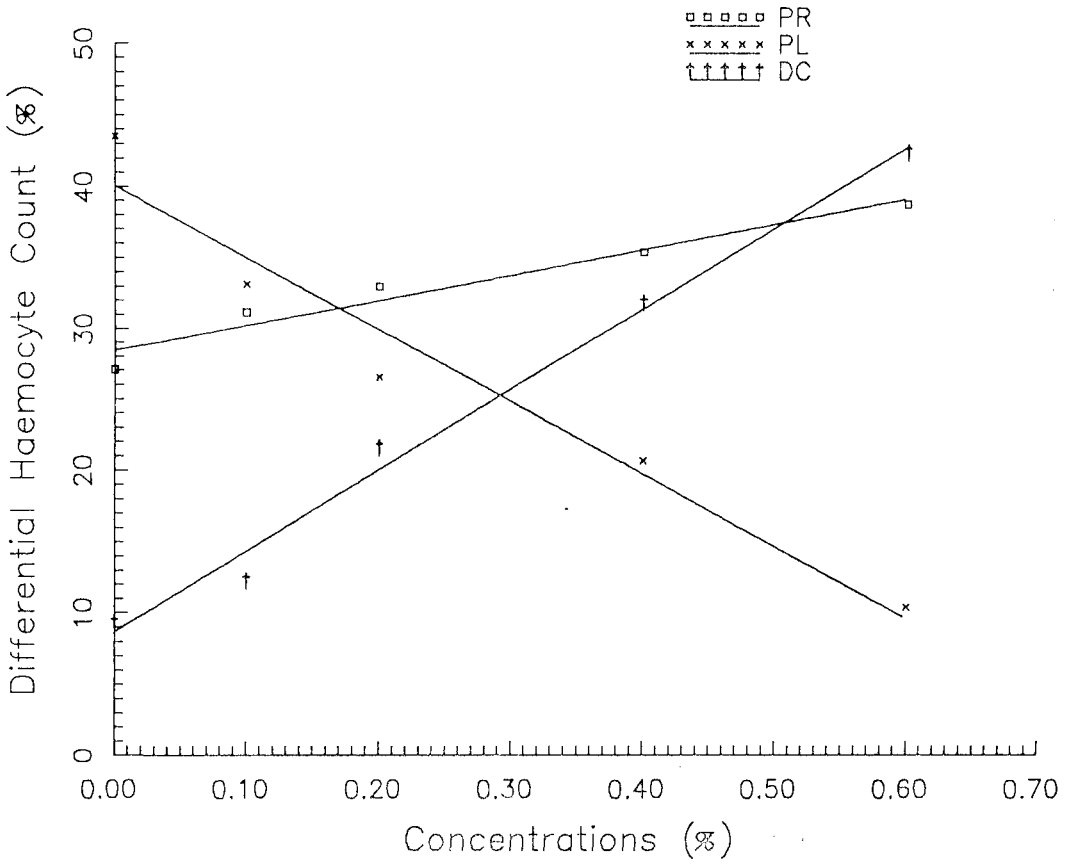

Fig. 27 : Correlation between the Differential Haemocyte Count (%) and various concentrations of aminocarb after 5 days of the treatment on one day old 6th instar larvae of Diacrisia obliqua.

significantly higher (38.76%, t= 2.803, P<0.05) in the blood smears affected with the highest concentration compared to control (27.04%). Plasmatocytes showed significant reduction in population by 0.4 and 0.6% aminocarb (t=3.454 and t=4.463, P<0.05), respectively, as compared to control. The regression between concentration strength and plasmatocytes population yielded negative linear correlation (Y = 40.048 - 50.83 X, r = - 0.9773, P<0.001). Alterations in the population of other haemocyte types were statistically insignificant at 5% level. However, population of disintegrating cells linearly increased from 9.26% in control to 12.26% (t= 1.654, P>0.05), 21.56% (t=3.167,P<0.05), 31.82% (t= 4.878, P<0.05) and 42.38% (t=5.488, P<0.05), respectively, following treatment with progressively higher concentration of aminocarb, and was significant at all concentrations except at the lowest (Y = 8.662 + 56.90 X, r = 0.9951, P<0.001).

After pupal ecdysis (Table-50)

In the pupal stage, the DHCs were determined only by three concentrations because the treatment with the highest concentration (0.6%) caused heavy mortality in the 6^{th} instar which resulted in the non-availability of the pupae. Prohaemocytes population was although high in 0.1, 0.2 and 0.4% aminocarb affected pupae, it, however was statistically insignificant. The plasmatocytes were significantly less at 0.2% (49.78%, t= 3.290, P<0.05) and at 0.4% (42.04%, t= 4.03, P< 0.05) aminocarb compared to control (73.92%). Granulocytes constituted 2.42% of total haemocytes in the control, but at 0.1, 0.2 and 0.4% aminocarb their population became 3.50, 3.4 and 4.12% respectively. However, the increase was insignificant statistically at 5% level. Spherulocytes and oenocytoids were altogether absent in control as well as treated pupae. Coagulocytes were sporadically present. The population of disintegrating haemocytes was 9.26% in control. It increased to 11.54% (t= 1.358, P>0.05), 20.24% (t= 3.249, P<0.05) and26.78% (t= 4.027, P<0.05) respectively, as compared to control.

3. Effect of topical application of different concentrations of Cypermethrin (a pyrethroid) on *Diacrisia obliqua*.

When two-day-old 6th instar larvae were treated with 0.0025, 0.005, 0.01 and 0.015% cypermethrin, the respective mortalities in first 24 hrs were approximately 28-35%, 50-53%, 75-80% and 90-94%. Moreover, larvae treated with the highest concentrations could not successfully transform into pupae. The pharmacological symptoms were concentration based and were almost similar in nature and extent to those appeared in case of acephate and aminocarb.

3.1 Haemocyte picture of 6th instar larvae and pupae of *Diacrisia obliqua* affected with different concentrations of Cypermethrin.

As described earlier, in case of acephate and aminocarb, the blood picture of larvae treated with various concentrations of cypermethrin was studied after 6 hrs, 1 day, 3 days and 5 days following the application. Moreover, blood picture of affected 1-day-old pupae was also scrutinized.

The haemocytes did not show any significant pathological symptoms after 6 hrs of application of 0.0025 and 0.005% cypermethrin. Whereas application of 0.01 and 0.015% cypermethrin produced vacuolization of cytoplasm in some plasmatocytes and spherulocytes. Prohaemocytes oenocytoids and coagulocytes were unaffected morphologically although their relative population was sometimes significantly or insignificantly altered (Plate-XII, Fig. A & B).

After one day of treatment with lower concentrations (0.0025 and 0.005% cypermethrin), some plasmatocytes showed vacuolization in their cytoplasm, irregular plasma membrane and occasional nuclear distortion etc. (Plate-XII, Fig. C). However higher concentrations caused more intense vacuolization of cytoplasm, distortion of cell shape and shrinkage in cell size. Disruption of and decrease in visibility of cytoplasmic structures were observed in majority of plasmatocytes. As the active

degenerative changes progressed, the normal cytoplasmic structure became disrupted or assumed an abnormal appearance. As degeneration proceeded many of the affected cells became relatively achromophilic, particularly nuclei of these cells. Spherulocytes showed discharge of spherules into the surrounding blood. Prohaemocytes, largely developed gross abnormalities in the nucleus, represented by achromophilia, clumping of chromatin, distorted shape, and ragged appearance (Plate-XII, Fig. D). Cytoplasm of some prohaemocytes had a number of broad bulges, some times cell formed a greater number of smaller bulges or pseudopodia which gave the peripheries of the cells a very irregular appearance. Oenocytoids were slightly deformed. Severely affected granulocytes could not be identified clearly because of vacuolization of the cytoplasm.

After 3 days following the application of lower concentrations (0.0025 and 0.005%) of this chemical, haemocytes showed regenerative changes, which were also evident by the appearance of fusiform plasmatocytes and apparently normal prohaemocytes. Some plasmatocytes exhibited moderate vacuolization, loss of structural organization and achromophilia. No apparent degenerative changes were observed in oenocytoids and coagulocytes (Plate-XII, Fig. E & F). In the blood smears affected with the higher concentrations (0.01 and 0.015%) advanced cellular degeneration was observed which was more severe than that appeared in blood smears after 1 day of application. The affected prohaemocytes largely showed nuclear degeneration represented by breaking up of nuclei into a number of discrete bodies, vacuolization, swelling, distorted shape and achromophilia. The cytoplasmic abnormalities mostly included formation of bulges, or small tentacles giving the cells an irregular appearance and discharge of cytoplasm. However, a large number of prohaemocytes were apparently normal except showing smaller cell size compared to control. These were rather young cells and were perhaps freshly released into the haemolymph. Plasmatocytes were most susceptible cells and exhibited all kinds of cytoplasmic and nuclear abnormalities. Most of these cells were damaged beyond recognition. Even though majority of plasmatocytes showed histopathological abnormalities, a fair number of these cells appeared morphologically normal. Spherulocytes and granulocytes were damaged beyond recognition. Mitosis was frequently encountered and all stages of mitotic division were observed even in the

highly affected haemocytes. The blood plasma became very thick due to gelation and probably due to water loss during vomiting and diarrhea resulted in the treated larvae with higher concentrations. Since plasma became thick in consistency the haemocytes did not spread homogeneously during smear making therefore several highly concentrated haemocyte areas were consequently formed. These haemocytes showed shrinkage in cell size as well as overlapping which posed hindrance in critical observations.

After 5 days following 0.0025 and 0.005% cypermethrin treatment, the haemocytes in general, did not show pathological symptoms except occasional distorted haemocytes and clumping of cells. However, following application of 0.01% cypermethrin, cell changes, in general, included some cell agglutination, distortion and disintegration (Plate-XII, Fig. H). Like previous observations (i e blood picture after 3 days) plasmatocytes suffered maximum histopathological damage. Majority of them lost their fusiform shape and many cells became distinctly rounded, oval and flattened. Subsequently they showed a tendency to swell and became achromophilic, the cytoplasm became excessively vacuolated. The nucleus of majority of plasmatocytes showed distortion, fragmentation and extrusion. Prohaemocytes and oenocytoids were not noticeably affected. Granulocytes and spherulocytes were not distinguishable because of vacuolization of cytoplasm. Coagulocytes showed discharge of cytoplasm into the surroundings.

Following the highest concentration of cypermethrin extreme degenerative changes occurred in haemocytes. Haemolymph was highly viscous due to gelation of plasma and loss of water during vomiting and diarrhea. The larvae from which haemolymph was taken were nearly dehydrated, feeble and contained very little blood. The haemolymph smears of these larvae showed clumping of cells of all kinds to a large extent. Usually clumps were composed of masses of all kinds of cells. Often, however, agglutination of similar cells occurred. These cells were small in size than the control and were either over stained or showed achromophilia. The distinction between cytoplasm and nucleus was somewhat lost. Spherulocytes, granulocytes and coagulocytes were somewhat indistinguishable. Identity of most of the plasmatocytes

was also lost because of extreme cytoplasmic and nuclear degeneration. Prohaemocytes and oenocytoids were still recognizable (Plate-XII, Fig. I).

The larvae treated with 0.01 and 0.015% cypermethrin could not successfully transform into pupae. Therefore blood picture of only 0.0025 and 0.005% cypermethrin affected pupae was observed. The smears of these pupae contained apparently normal haemocytes. However, some plasmatocytes showed irregular shape as well as cytoplasmic discharge (Plate-XII, Fig. G).

3.2 THCs of 6th instar larvae and pupae of *Diacrisia obliqua* affected with different concentrations of Cypermethrin.

The total haemocyte counts determined in the treated larvae at the different time intervals are summarized in table-38.

After 6 hrs

After 6 hrs of treatment, the two lower concentrations of cypermethrin brought about an increase in the total haemocyte counts. On the other hand higher concentration of cypermethrin viz 0.01 and 0.015% resulted in 41.93 and 70.82% reduction which was significant at 10% level ($t=2.395$ and $t= 2.653$, $P<0.10$).

After 1 day

The THCs/mm^3 of untreated and acetone treated larvae (control) of corresponding age and stages were found to be 44300 ± 7695.13 and 42680 ± 6226.41, respectively. After 24 hrs of application of the lower concentrations (0.0025 and 0.005%) the number of haemocytes per cubic mm blood was increased by 39.83% and 49.32%, respectively. compared to control, which was statistically insignificant. Similarly as mentioned above, the higher concentrations (0.01 and 0.015%) reduced the cell population by 35.80 and 77.16% in comparison to control.

After 3 days

The THC of untreated and acetone treated 6th instar larvae of corresponding age were found to be 66590 ± 10100.77 and 60850 ± 8700.82 cells/mm^3, respectively. The total counts of larvae affected with 0.0025% and 0.005% cypermethrin were slightly and insignificantly higher compared to control whereas 0.01 and 0.015% cypermethrin dropped cell population by 47.94% (t=2.306, P>0.05) and 85.71% (t=3.260, P<0.05), respectively, in comparison to control.

After 5 day

The total counts were insignificantly higher by the lowest concentration (t= 1.016, P>0.05). The remaining three concentrations viz. 0.005, 0.01 and 0.015% cypermethrin caused progressive reduction of 12.10% (t=1.526), 53.70 % (t= 3.225) and 81.99% (t= 3.639) in the total haemocyte counts as compared to control. The untreated and acetone treated larvae contained 61850 ± 5589.57 and 59980 ±3684.79 cells /mm^3 of haemolymph, respectively.

After pupal ecdysis

The THCs of individual pupa of untreated larvae, control as well as that of treated larvae showed a great variation thereby resulting in a high standard error value. The total cell counts of untreated and control larvae were found to be 9440 ± 1582.59 and 10860 ± 3029.99 cells/mm^3 of blood respectively. The application of the lowest concentration (0.0025%) on 6th instar larvae enhanced the THC by 11.05%. However the treatment of 6th instar larvae with 0.005% cypermethrin caused 18.05% reduction. Following the application of the two higher concentrations (0.01 and 0.015%) the THC could not be determined in the pupae as the affected larvae died before reaching the pupal stage.

3.3 DHCs of 6th instar larvae and pupae of *Diacrisia obliqua* affected with different concentrations of Cypermethrin.

The DHCs of 6th instar larvae were calculated after 6 hrs, 1 day, 3 day, and 5 days following topical application of 0.0025, 0.005, 0.01 and 0.015% cypermethrin on 2-day-old 6th instar larvae. Moreover, when the treated larvae moulted to pupal stage, DHC of 1 day old affected pupae were also determined. The relative percentage of different types of haemocytes at various time intervals was as follows.

After 6 hrs (Table-51, Fig. 28)

Prohaemocyte population showed slight and statistically insignificant increase following treatment with all the selected concentrations of cypermethrin. Regression between plasmatocyte population and concentration strength of cypermethrin yielded a negative linear correlation ($Y= 50.49 - 2661.17 X$, $r = - 0.9791$, $P<0.001$). The plasmatocyte population fell to 18.58% following 0.01% cypermethrin application and 13.24 by 0.015% cypermethrin compared to control (49.32%), thus showing a statistically significant reduction ($t=3.650$, $t=4.183$, $p<0.05$ respectively). Granulocytes exhibited an inconsistent alteration in population. Spherulocytes were absent in the smears affected with 0.01 and 0.015% cypermethrin. Oenocytoids although exhibited an increase in population, it, however, was statistically insignificant because of very high variation in population. Coagulocytes showed an increase in population with respect to cypermethrin treatment. The unidentified and disintegrated cells had a positive linear correlation with increase in concentration ($Y= 8.357 + 1809.66 X$, $r = 0.9520$, $P<0.001$). The increase was statistically significant by 0.01 and 0.015% cypermethrin ($t= 3.976$, $t= 3.493$ respectively, $P<0.05$).

After 1 day (Table-52, Fig. 29)

After 1 day of treatment with increasing concentrations, prohaemocytes showed an increase in population. Plasmatocytes on the other hand, were significantly decreased to 27.44% ($t=3.820$, $P<0.05$), 17.32% ($t=4.236$, $P<0.05$) and 5.10% ($t=5.156$, $P<0.05$) compared to control (51.52%). Regression between concentration

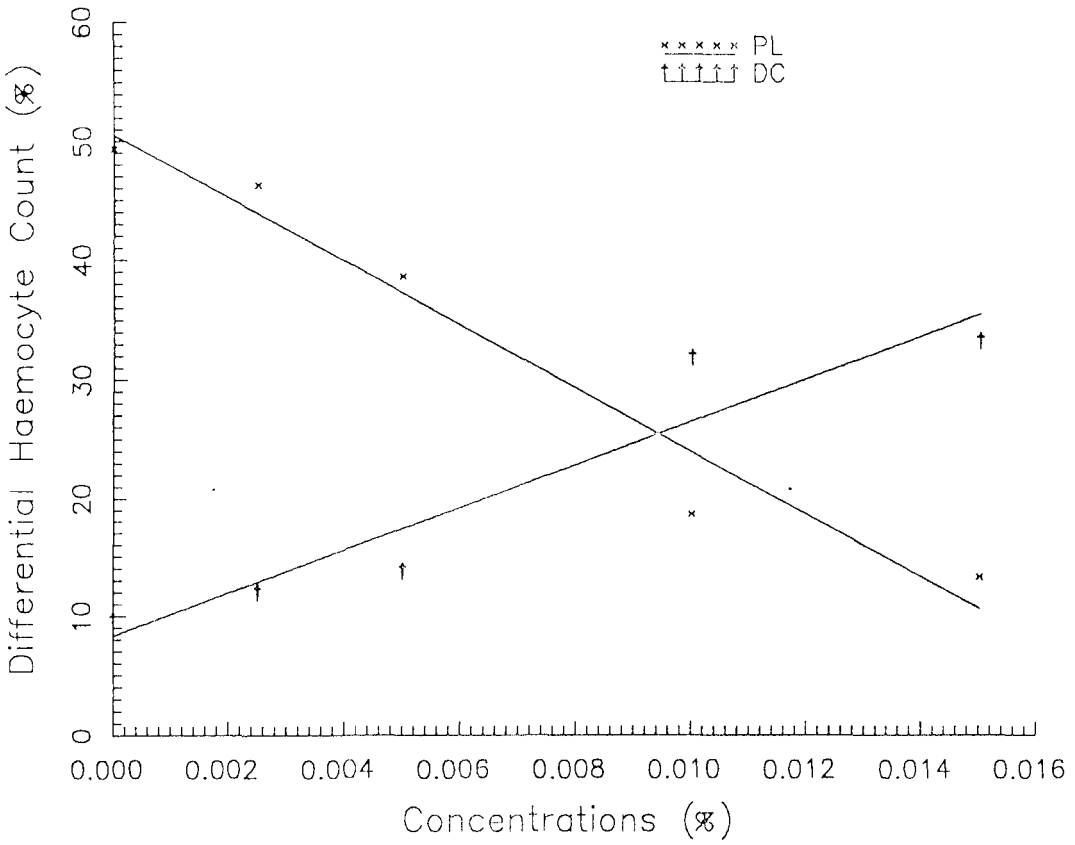

Fig. 28 : Correlation between the Differential Haemocyte Count (%) and various concentrations of cypermethrin after 6 hrs of the treatment on one day old 6th instar larvae of Dinarisia obliqua.

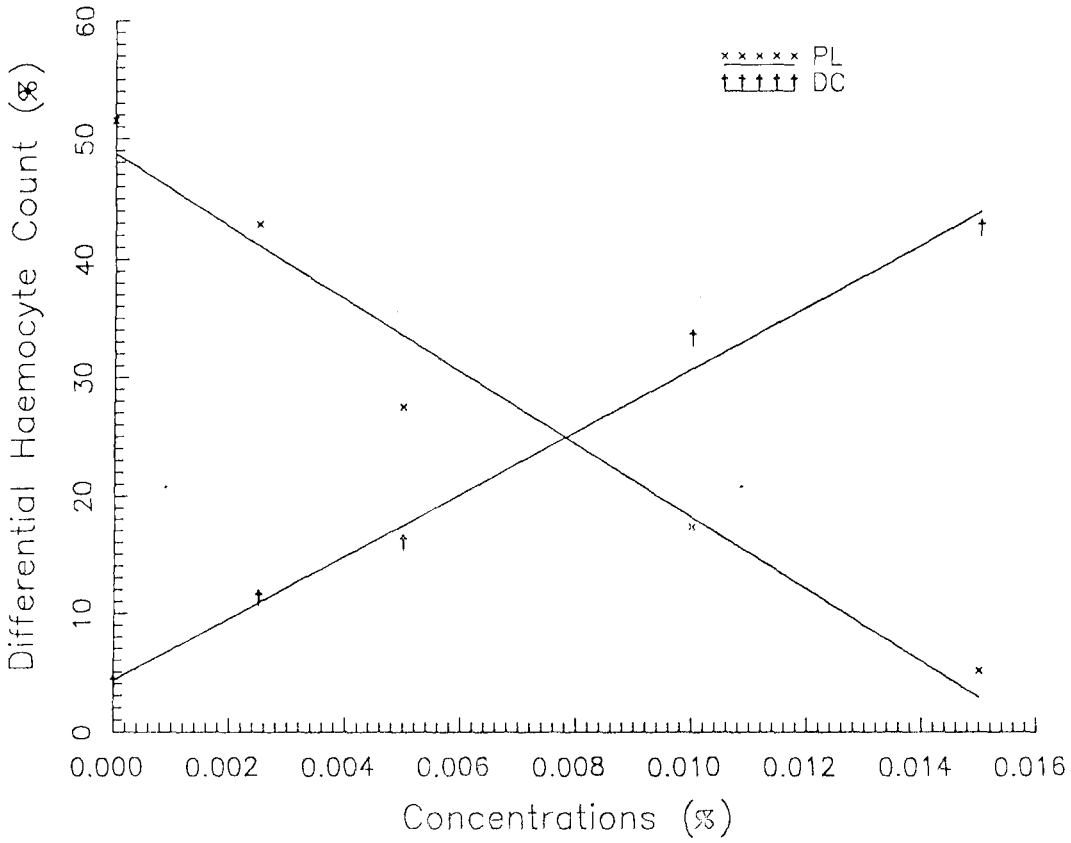

Fig. 29 : Correlation between the Differential Haemocyte Count (%) and various concentrations of cypermethrin after one day of the treatment on one day old 6th instar larvae of Diacrisia obliqua.

strength and plasmatocyte population yielded a negative linear correlation (Y = 48.72 - 3058.14 X, r = - 0.9812, P<0.001). Granulocytes and spherulocytes were absent from the smear after 0.01 and 0.015% cypermethrin application. Oenocytoid population showed a fourfold increase at the highest concentration which was statistically significant (t=2.920). The disintegrating and unidentified cells were significantly increased to 11.30% (t= 3.468, P<0.05), 16.04%(t=3.533, P<0.05), 33.26% (t=4.424, P<0.05), and 42.46% (t=5.861, P<0.05) following treatment with 0.0025, 0.005, 0.01 and 0.015% cypermethrin. Furthermore, the regression between concentration strength and population of disintegrating cells yielded a strong positive linear correlation (Y= 4.32 + 2629.86 X, r = 0.9945, P<0.001).

After 3 days (Table-53, Fig. 30)

Prohaemocyte population exhibited a progressive increase with respect to 0.0025, 0.005 and 0.01% cypermethrin which was significant at 0.01% concentration (t=3.178, P<0.05). Plasmatocyte population showed a negative linear correlation with increase in concentration strength (Y= 54.30 - 2728.14 X, r = - 0.9926, P<0.001). The reduction in plasmatocyte population was significant at 0.01 and 0.015% cypermethrin (t=3.751,and t=4.926 respectively, P<0.05). Granulocytes and spherulocytes were completely damaged in smears affected with all concentrations except the lowest. Oenocytoids and coagulocytes exhibited an increase in population with respect to increasing concentrations of cypermethrin, which, however, was statistically insignificant. On the other hand, the population of disintegrating and damaged cells showed a positive linear correlation with increasing concentrations of cypermethrin (Y= 10.16 + 1344.34 X, r = 0.9295, P<0.001). The increase in population of these haemocytes was statistically significant at 0.005, 0.01 and 0.015% cypermethrin (t= 3.223, t=3.407 and t= 3.565 respectively, P<0.05) as compared to control.

After 5 days (Table-54, Fig. 31)

The prohaemocyte population increased to 34.04%, 35.52%, 38.38% and 39.60% after 5 days of treatment with 0.0025, 0.005, 0.01 and 0.015% cypermethrin showing positive linear correlation (Y= 31.37 + 620.69 X, r = 0.9367, P<0.001) in

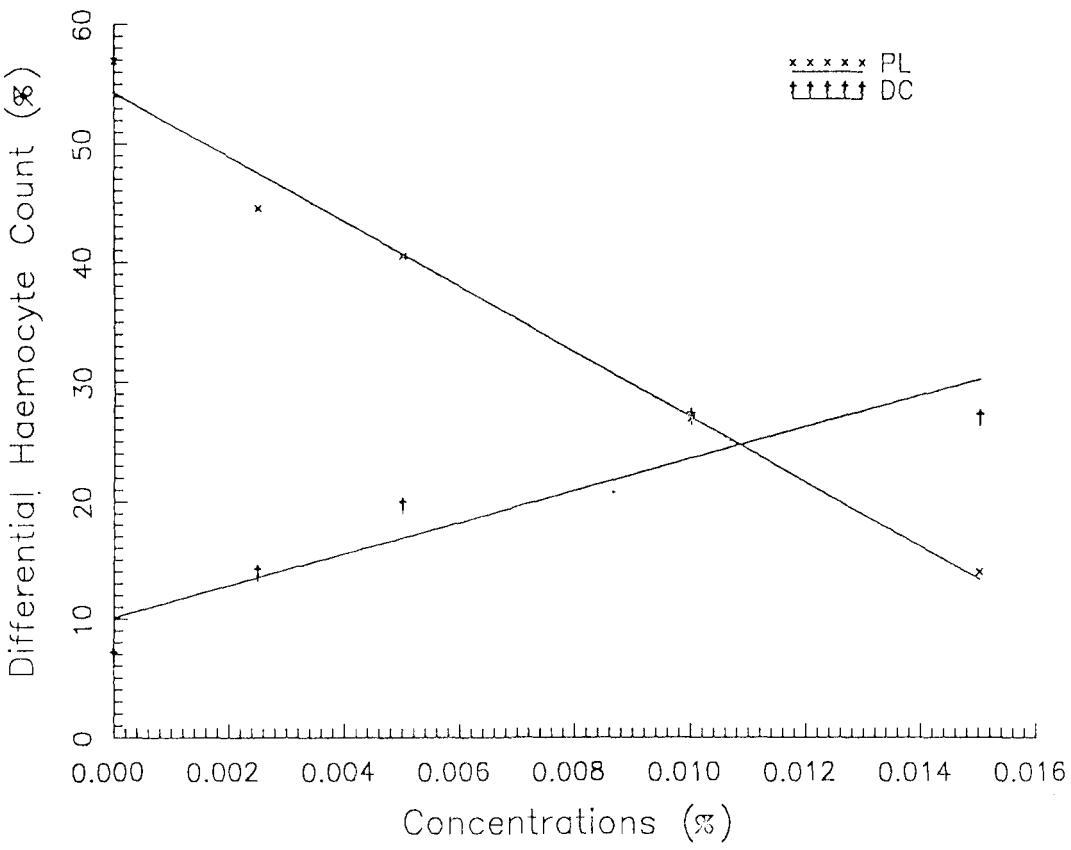

Fig. 30 : Correlation between the Differential Haemocyte count (%) and various concentrations of cypermethrin after 3 days of the treatment on one day old 6th instar larvae of Diacrisia obliqua.

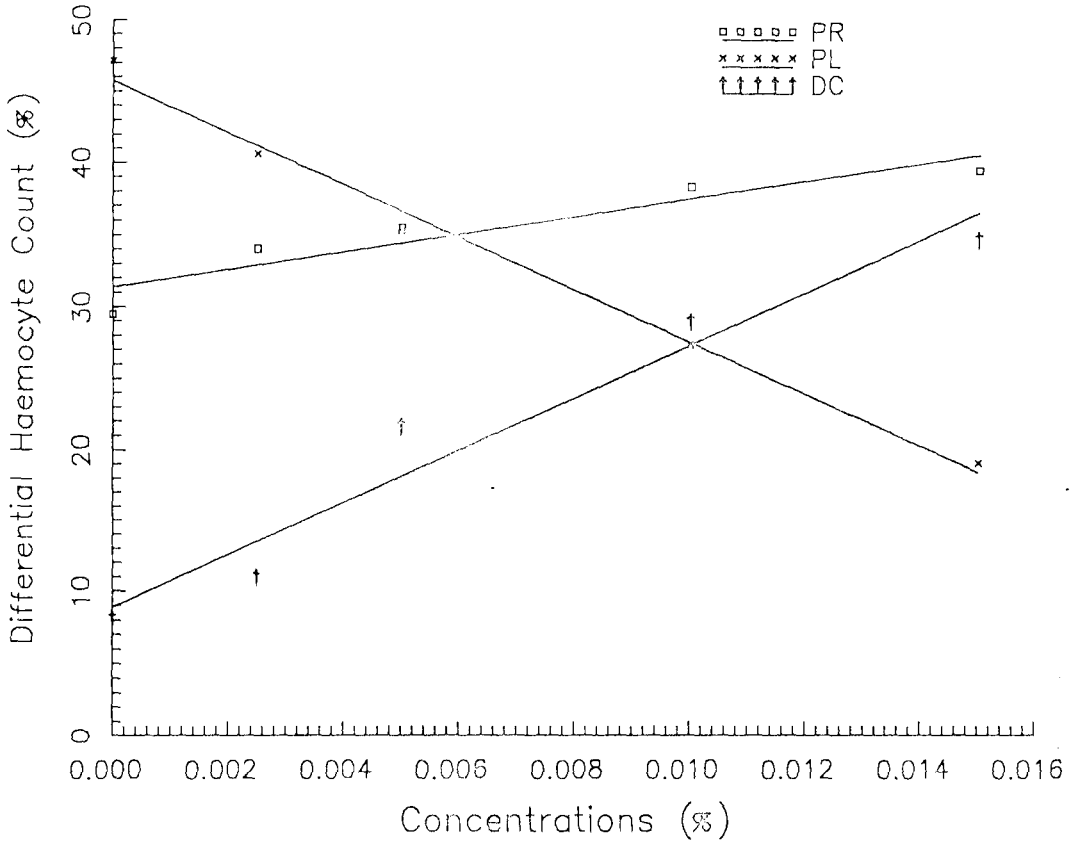

Fig. 31 : Correlation between the Differential Haemocyte Count (%) and various concentrations of cypermethrin after 5 days of the treatment on one day old 6th instar larvae of Diacrisia obliqua.

comparison to control (29.46%). The increase was significant at the highest concentration (t= 2.799, P<0.05). On the other hand, plasmatocyte population showed linear reduction with respect to increasing concentrations of cypermethrin (Y= 45.71 - 1813.07 X, r = - 0.9953, P<0.001) which was significant by the highest concentration (t=3.063. P<0.05), whereas, granulocytes and spherulocytes were completely damaged at higher doses. There was an apparent increase in relative population of oenocytoids that might be due to disintegration of other types of haemocytes (because their actual number in the smear did not increase much in comparison to control). The population of disintegrating cells increased to 10.96% (t=3.692, P<0.05), 21.66% (t=3.692, P<0.05), 29.0%(t=3.965, P<0.05) and 34.82% (t= 3.970, P<0.05) following the treatment with 0.0025, 0.005, 0.01 and 0.015% cypermethrin, respectively. The regression between concentration strength and damaged cell population yielded a positive linear correlation (Y = 08.853 + 1853.45 X, r = 0.9753, P<0.001).

After pupal ecdysis (Table-55)

In one-day-old pupae, spherulocytes, oenocytoids and coagulocytes were absent with respect to treated as well as control larvae. The prohaemocytes were 16.72 and 20.72% of the total cell population in larvae affected with 0.0025 and 0.005% cypermethrin compared to 14.28% in the control. Plasmatocytes constituted 68.60 and 57.58% (t=2.337, P>0.05 and t=3.945 P<0.05, respectively) of total haemocytes affected with 0.0025 and 0.005% cypermethrin compared to 76.50% in control. The reduction was significant at 0.005% cypermethrin. Granulocytes showed an insignificant increase at the two above-mentioned concentrations. Similarly, disintegrating cells showed an insignificant increase in population compared to control.

4. Effect of topical application of different concentrations of Muristerone (a phytoecdysone) on *Diacrisia obliqua.*

When different concentrations viz 0.5, 1.0, 1.5 and 2.0% muristerone were applied on 2 day old 6th instar larvae of *D. obliqua*, the respective mortalities were

15-20%, 25-32%, 36-41% and 46-50% in the 6th instar larval stage. Feeding was not affected in the larvae treated with 0.5 and 1.0% muristerone and approximately 10-15% mortality was observed in prepupal stage. However, feeding was slow in larvae affected with higher concentrations. Regurgitation of greenish fluid and diarrhea was also observed but it was not as copious as was found in case of aforementioned three insecticides. With respect to the highest concentration, the treated larvae suffered heavy mortality at the time of larval-pupal moulting. Similarly, in case of 1.5% muristerone, when white covering meant for pupae were observed, they were found to contain prepupae which could not successfully transform into pupae.

4.1 Haemocyte picture of 6th instar larvae and pupae of *Diacrisia obliqua* affected with different concentrations of Muristerone.

The haemocyte picture of 6th instar larvae after 6 hrs, 1 day, 3 days and 5 days, following the application of various concentrations of muristerone, as well as in 1 day old affected pupae is given below.

After 6 hrs of treatment with 0.5, 1.0 and 1.5% muristerone, the haemocytes did not show any noticeable change in their morphology. However, the highest concentration (2.0%) induced slight vacuolization of cytoplasm and in some case nucleus also (Plate-XIII, Fig. B). Granulation of cytoplasm was also observed in many haemocytes. Prohaemocytes, although did not exhibit any adverse effect on cell morphology, their population increased to some extent and a large number of them appeared to be newly differentiated and freshly released into circulation. These prohaemocytes were smaller in size and found in aggregates of few to many cells. Oenocytoids and coagulocytes were rather unaffected (Plate-XIII, Fig. C). Plasmatocytes were moderately affected showing ragged appearance, spreading on glass surface, bulgings or tentacles on the cell surface and vacuolization of cytoplasm as well as nucleus. The nuclei of some cells became partially or completely extruded. Occasionally, groups of naked nuclei were also observed. Various phases of mitosis, early as well as advanced, were also detected in the smears.

One day following the treatment, there were almost similar pathological abnormalities as were observed 6 hrs post-treatment. Oenocytoids, coagulocytes and spherulocytes appeared normal in majority of smears (Plate-XIII, Fig. A). The plasmatocytes occasionally indicated a swollen appearance and in the areas of smears where cell population was very thin, these haemocytes appeared exceptionally large in size. Severe degenerative changes in haemocytes as found in cases of acephate, aminocarb and cypermethrin were not generally observed with muristerone. Clumping of haemocytes and non-homogeneous distribution of cells in the smears were not observed in the larvae affected with the highest concentration. Achromophilia in the nuclei of prohaemocytes and plasmatocytes was also occasionally observed.

After 3 days of application of higher concentrations, the cytoplasmic and nuclear deformities were spread to more haemocytes. The plasmatocytes were mostly round or oval, generally containing numerous small vacuoles and occasionally one or two large vacuoles in their cytoplasm (Plate-XIII, Fig. F). Moreover, surface activity in the form of bulges or very thin lamellar cytoplasmic extensions, as well as a single large axon like extension was also evident in some plasmatocytes. The nuclear abnormalities were characterized by the achromophilia, distorted shape and fragmentation. Spherulocytes were not distinguishable in the smears affected with two higher concentrations (1.5 and 2.0%). In addition, many intermediate cells were also present in the smears that showed cytoplasmic as well as nuclear deformities. Following the lower concentrations (0.5 and 1.0% muristerone) blood picture did not alter noticeably in comparison to control.

Three days following the treatment with the highest concentration (2.0%), the larvae suffered heavy mortality because of early onset of moulting under the influence of hormone application. Thus larvae were not available for observations 5days post-treatment. After five days following treatment with 1.5% muristerone, spherulocytes became indistinguishable, granulocytes showed slight vacuolation of cytoplasm and discharge of granules from the cells. Whereas, coagulocyte population seemed to be relatively more in these smears. Plasmatocytes exhibited cytoplasmic and nuclear abnormalities, similar to those mentioned before. Oenocytoids and Prohaemocytes were unaffected (Plate-XIII, Fig. E).

The 6th instar larvae treated with 1.5% muristerone could not successfully transform into pupae. Therefore, haemocytes were studied only in the pupae affected with 0.5 and 1.0% muristerone (Plate-XIII, Fig. G & H). Only plasmatocytes displayed significant damage and disintegration of cellular structure. Other haemocytes viz. Prohaemocytes, granulocytes and coagulocytes appeared almost normal.

4.2 THCs of 6th instar larvae and pupae of *Diacrisia obliqua* affected with different concentrations of Muristerone.

As mentioned before, the THCs of 6th instar larvae were determined after 6 hrs, 1 day, 3 days and 5 days following treatment with various selected concentrations. THCs of 1-day-old pupae were also determined. The age-wise mean THCs are summarized in table-39.

After 6 hrs

The THCs showed an increasing trend with three lower concentrations (viz., 0.5, 1.0 and 1.5%). Only the highest concentration (2.0%) caused a reduction ((24.77%) in the total cell counts as compared to control. However, all the alterations in THCs were insignificant at 5% level.

After 1 day

Muristerone had a stronger effect on the total cell count after 1 day of its application. The three lower concentrations viz 0.5, 1.0 and 1.5% caused an increase of 17.58%, 24.78% and 32.89% as compared to control. On the other hand, the highest concentration reduced the THC by 20.01% that, however, was statistically insignificant.

After 3 days

The THCs of untreated and acetone treated 6th instar larvae of corresponding age were found to be 69070 ± 4332.28 and 70320 ± 5427.33 cells/mm^3 of haemolymph. The two lower concentrations enhanced the cell counts by 20.80% and 23.82% respectively as compared to control. On the contrary, the two higher concentrations resulted in 14.29% and 36.70% reduction in THC as compared to control. The highest concentration induced a significant reduction in THC at 10% level of significance.

After 5 days

After 5 days of application, 1.0% concentration of muristerone induced 28.17% ($t=1.190$, $P>0.05$) increase in THC but 1.5% muristerone resulted in 11.49% reduction ($t=2.152$, $P>0.05$) as compared to control. The untreated and control larvae exhibited a total count of 50260 ± 2149.85 and 51370 ± 1327.93 cells/mm^3 respectively. Following the application of the highest concentration, THC could not be recorded after 5 days due to unavailability of larvae of the corresponding age.

After pupal ecdysis

One day old pupae of untreated and control larvae contained 10820 ± 1519.01 and 10970 ± 1815.74 cells /mm^3 of haemolymph. Whereas the pupae affected with 0.5 and 1.0% muristerone, the population of total haemocytes was enhanced by 20.78 and 38.65% of the control.

4.3 DHCs of 6th instar larvae and pupae of *Diacrisia obliqua* affected with different concentrations of Muristerone.

The population of different types of haemocytes was also determined after 6 hrs, 1 day, 3 days and 5 days of hormonal treatment. When the treated larvae moulted

to pupal stage, the differential counts were made in one-day-old pupae. The differential counts at various selected time intervals were as follows.

After 6 hrs (Table-56, Fig. 32)

The prohaemocyte and plasmatocyte population exhibited a declining trend compared to their population in the control. Granulocyte population showed a concentration-based increase. With respect to the highest concentration, it became approximately double of the control, however, it was insignificant at 5% level of significance. Furthermore, the regression between dose strength and granulocyte population exhibited a positive linear correlation ($Y= 2.98 + 2.11 X$, $r = 0.9897$, $P<0.001$). Spherulocytes did not show any appreciable alteration in the hormone-affected larvae. Oenocytoids were significantly increased at the highest concentration. Coagulocytes, although, were slightly enhanced in the treated larvae, however, they showed inconsistency with respect to increasing concentrations of muristerone. Moreover, following treatment of the highest selected concentration (2.0%) these haemocytes were absent from the smear. The population of damaged and unidentified haemocytes showed a consistent and concentration based significant increase in their percentage as compared to control. The relative percentage of these cells was found to be 9.48% ($t= 2.693$, $P>0.05$), 11.38% ($t= 3.274$, $P<0.05$), 19.88% ($t= 3.747$, $P<0.05$) and 22.22% ($t= 3.717$, $P<0.05$) following treatment with 0.5, 1.0, 1.5 and 2.0% muristerone, respectively, compared to control. The regression between concentration strength and population of disintegrating cells yielded a positive linear correlation ($Y= 4.72 + 8.90 X$, $r = 0.9788$, $P<0.001$).

After one day (Table-57, Fig. 33)

Prohaemocytes were insignificantly increased by all the concentrations of muristerone. However, plasmatocytes exhibited a concentration-based reduction in their population, which was statistically significant with respect to 1.5 and 2.0% muristerone. The regression between concentration strength and plasmatocyte population yielded a negatively linear correlation ($Y= 49.61 - 13.23 X$, $r = - 0.9975$, $P<0.001$). On the other hand granulocytes showed a concentration based linear

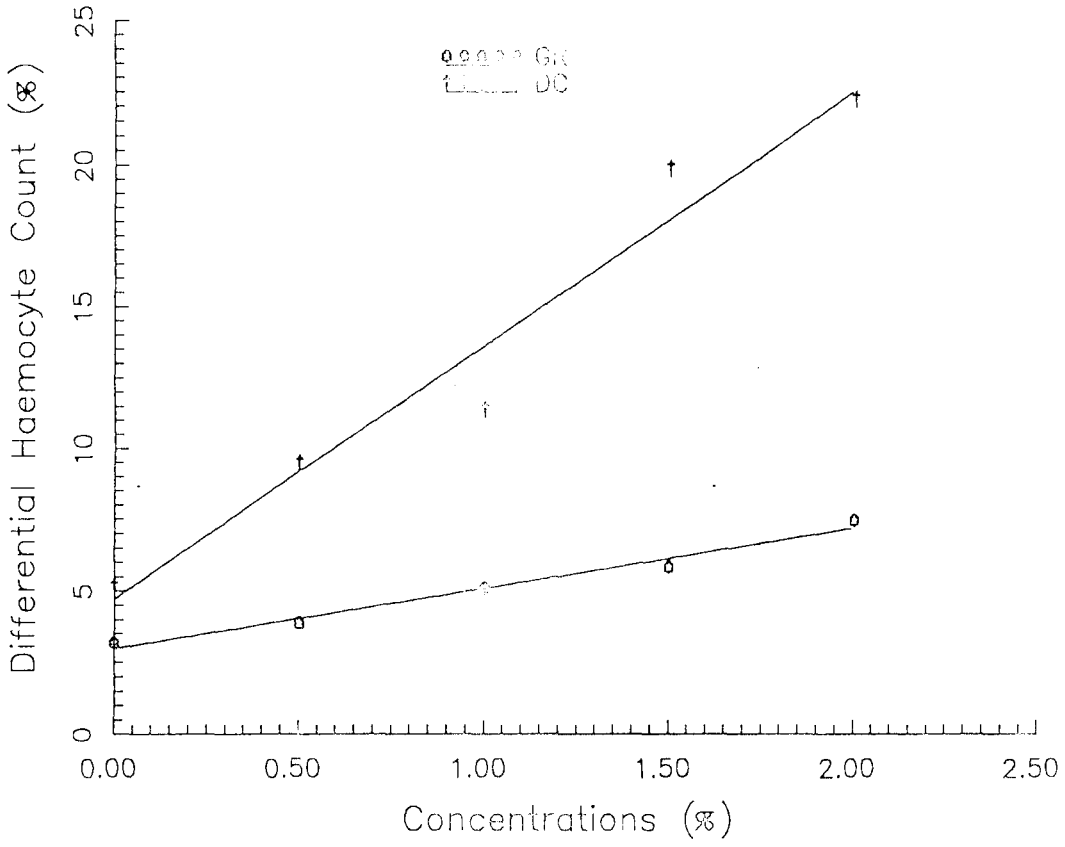

Fig. 32 : Correlation between the Differential Haemocyte Count (%) and various concentrations of muristerone after 6 hrs of the treatment on one day old 6th instar larvae of Diacrisia obliqua.

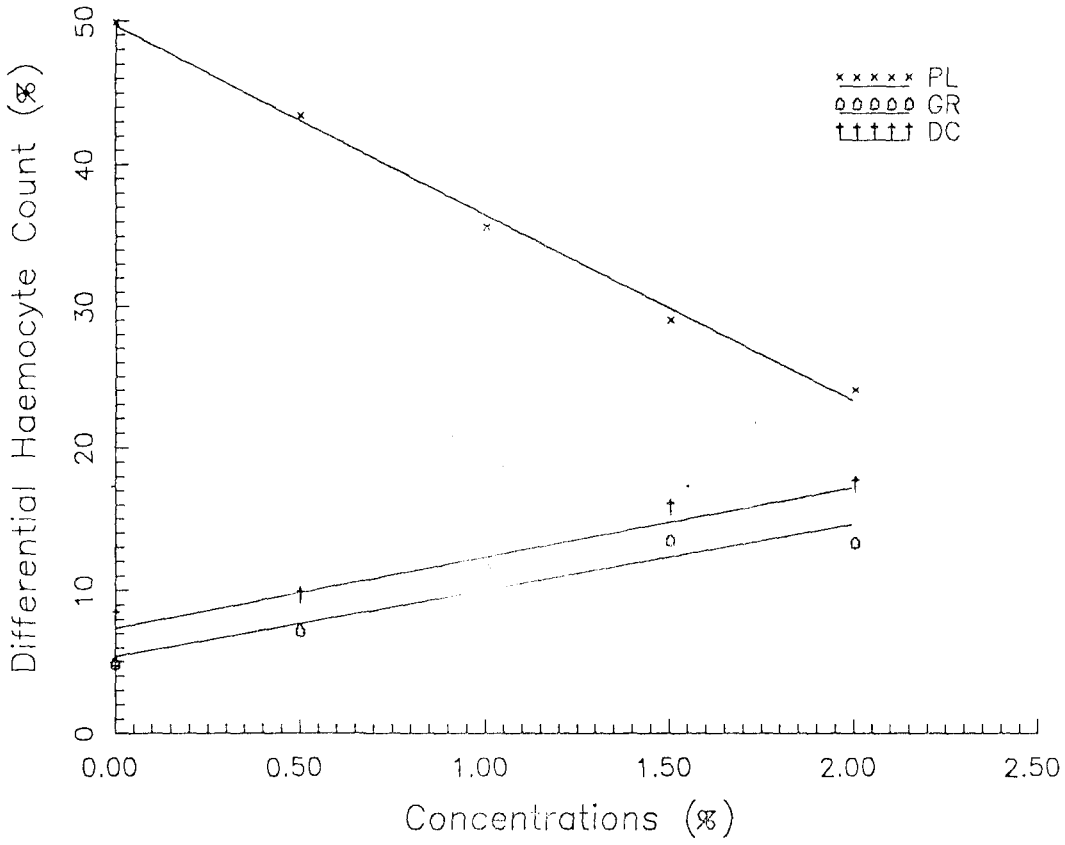

Fig. 33 : Correlation between the Differential Haemocyte Count (%) and various concentrations of muristerone after one day of the treatment on one day old 6th instar larvae of Diacrisia obliqua.

increase in their population (Y = 5.4 + 4.61 X, r = 0.9509, P<0.001) which was significant at 1.5 and 2.0% muristerone (t=3.031 and t=2.949 respectively, P<0.05). Spherulocytes could not be identified in the smears affected with the above mentioned concentrations. Oenocytoids of affected larvae were only very slightly enhanced compared to control. Coagulocytes, although, showed an increase in percentage in the affected smears, it was statistically insignificant at 5% level. In the same way, disintegrating cells increased linearly in population (9.66%, 10.3%, 15.82% and 17.44%) and showed a positive correlation (Y= 07.38 + 04.91 X, r = 0.9541, P<0.001) with respect to hormonal treatment (0.5, 1.0, 1.5 and 2.0% muristerone), however, their population was quite high in the control (8.24%).

After 3 days (Table-58, Fig. 34)

As observed in the larvae after 1 day of the application of muristerone, the prohaemocytes still showed an increase in their relative percentage but it was insignificant at 5% level. Plasmatocytes were 52.14% in the control. Following the application with 0.5, 1.0, 1.5 and 2.0% muristerone their proportion was reduced to 49.56, 42.38, 31.46%, respectively, which was highly significant at two higher concentrations (t= 3.992 and t=4.203 respectively, P<0.05) and yielded a negative linear correlation (Y = 54.37 - 14.05 X, r = - 0.9827, P<0.001). Spherulocytes were totally absent at 1.5 and 2.0% muristerone. Oenocytoids, although exhibited a concentration based increase, it however, was statistically insignificant at 5% level. Unidentified and disintegrating cells were significantly high (15.92%, t= 3.280 and 21.6%, t= 3.68 respectively, P<0.05) in the larvae affected with 1.5 and 2.0% muristerone and showed a positive linear correlation (Y = 4.03 + 07.89 X, r = 0.9427, P<0.001).

After 5 days (Table-59)

The differential haemocyte counts were determined 5 days following treatment with various concentrations of muristerone. A severe histopathological damage was observed in 16.56% (t= 3.476, P<0.05) haemocytes (disintegrating cells) following the application of 1.5% muristerone. The DHCs of larvae affected with the highest

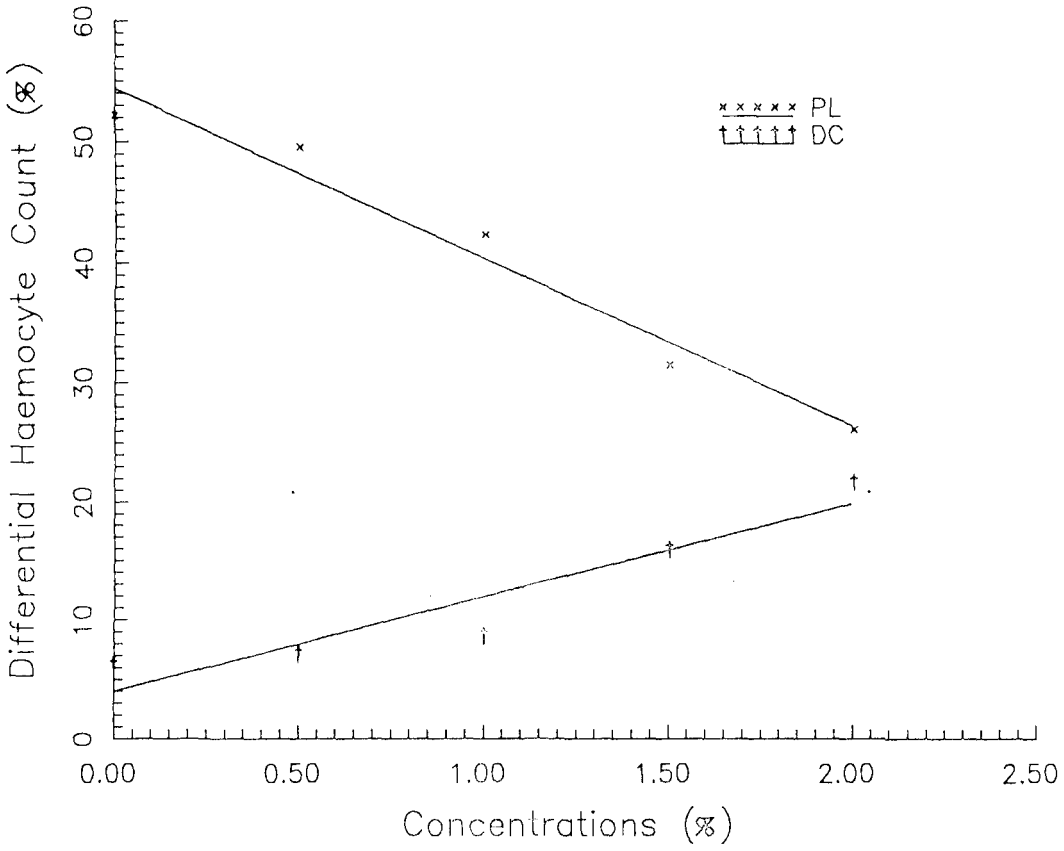

Fig. 34 : Correlation between the Differential Haemocyte Count (%) and various concentrations of muristerone after 3 days of the treatment on one day old 6th instar larvae of Diacrisia obliqua.

concentration (2.0%) could not be determined after 5 days of application since the insects were not available in that stage.

After pupal ecdysis (Table-60)

When the treated larvae moulted to the pupal stage, DHCs could be determined only in 0.5 and 1.0% muristerone affected batches because the two higher concentrations resulted in the formation of abnormal prepupae which could not successfully transform into pupae. Only plasmatocytes showed a significant reduction at 5% level ($t= 2.958$ $P<0.05$) when treated with 1.0% muristerone. Changes in the other haemocytes were statistically insignificant.

5. Effect of topical application of different concentrations of Methoprene (a juvenoid) on *Diacrisia obliqua*.

As mentioned in case of muristerone, the application of methoprene, too, did not cause heavy knock down of treated larvae within 48 hrs of application. The major cause of mortality in this case was abnormal moulting. Moreover, the larvae treated with 0.8 and 1.0% methoprene could not successfully transform into pupae. Other pharmacological symptoms were almost similar to those of muristerone treated larvae.

5.1 Haemocyte picture of 6^{th} instar larvae and pupae of *Diacrisia obliqua* affected with different concentrations of Methoprene.

Following the topical application of different selected concentrations of methoprene viz 0.4, 0.6, 0.8 and 1.0% on 6^{th} instar larvae of *Diacrisia obliqua* the permanent stained blood smears obtained after 6 hrs, 1 day, 3 days and 5 days of treatment were examined. The blood picture of mature pupae was examined wherever possible.

After 6 hrs of application of 0.4 and 0.6% methoprene the blood of treated 6th instar larvae contained apparently normal haemocytes except some plasmatocytes and spherulocytes which showed large cytoplasmic vacuoles (Plate-XIV, Fig. A). Small vacuoles were also formed in the cytoplasm of granulocytes as well as in prohaemocytes and oenocytoids. Following the application of higher concentrations more haemocytes were affected adversely, and a large population of about 30% haemocytes were distorted in such a way that their identity was lost. Maximum damage occurred in plasmatocytes. The mitotic figures were comparatively increased. Many prohaemocytes and plasmatocytes exhibited such nuclear picture, which was suggestive of very early prophase. Large chromatin granules were evident in these haemocytes.

After 1 day of application of 0.4 and 0.6% methoprene, the prohaemocytes and oenocytoids were not noticeably affected (Plate-XIV, Fig. B). Even though spherulocytes and granulocytes exhibited vacuoles in their cytoplasm as well as in nucleus, their population was increased as compared to control. The cell boundaries became irregular. In some cases spherulocytes showed discharge of spherules into the plasma. Plasmatocytes showed transformation in shape from fusiform to round or oval. Various stages of mitotic cell division were evident in the smear. No attempt was made to quantify these mitotic figures except in a few cases. The application of 0.8% methoprene had almost similar effect on haemocyte as observed with 0.6% methoprene. Following treatment with the highest concentration of methoprene, granules appeared in more haemocytes. The spherulocytes showed discharge of spherules into the plasma. Oenocytoids and prohaemocytes were not noticeably affected. Some plasmatocytes showed irregular cell membrane, appearance of small tentacles and bulges on the cell membrane, vacuoles in the cytoplasm and nucleus as well as fragmentation of nucleus etc. However, these cytoplasmic and nuclear abnormalities were confined to only a few plasmatocytes and spherulocytes (Plate-XIV, Fig. C).

After 3 days of treatment with 0.4 and 0.6% methoprene the general blood picture (Plate-XIV, Fig. D) did not alter much except granulocyte population. In some smears a large number of plasmatocyte like cells showed granules in cytoplasm. It

appeared that more haemocytes were differentiating into granulocytes. Coagulocyte population also was slightly more as compared to control. Other haemocytes appeared almost normal in size, shape and general morphology. Spherulocytes could not be identified in the blood smears. All stages of mitotic cell division, mostly in prohaemocytes and plasmatocytes, were present. The larvae treated with the highest concentration underwent heavy mortality at this stage as they prepared to undergo moulting.

Five days following the treatment with 0.4 and 0.6% methoprene, the haemocytes were not affected much. Only a small population of plasmatocytes showed appearance of moderate cytoplasmic and nuclear abnormalities characterized by distortion in shape of cell, vacuolization in the cytoplasm and nucleus, swelling of nucleus, as well as achromophilia etc. The treatment with 0.8% methoprene induced moderate pathological symptoms in some more plasmatocytes (Plate-XIV, Fig. E & F). Spherulocytes were not distinguishable in these smears. Prohaemocytes, granulocytes and oenocytoids were not adversely affected. The coagulocyte population was slightly more than the control. Smears contained many immature prohaemocytes, which appeared to be newly differentiated and freshly released into the smear. Besides that, there was an apparent increase in mitotic activity, which was evident in the nuclei of some cells by their assuming an aspect suggestive or definitely indicative of the early prophase of mitosis. Other phases of mitosis were also observed in blood smears from both normal and hormone affected larvae.

When the treated larvae moulted to pupal stage, heavy mortality occurred due to incomplete moulting which resulted in unviable larval-pupal intermediates. The apparently normal pupae were obtained when the treatment was made with 0.4 and 0.6% methoprene. The blood smears of these pupae showed a large population of plasmatocytes with moderate cytoplasmic and nuclear abnormalities (Plate-XIV, Fig. G).

5.2 THCs of 6th instar larvae and pupae of *Diacrisia obliqua* affected with different concentrations of Methoprene.

The account of THC of affected larvae at various time intervals is given below and also summarized in table-40.

After 6 hrs

The THCs of untreated and control larvae were recorded as 30870 ± 2989.38 and 31870 ± 3454.73 per mm^3 of haemolymph. There was an increase of 6.34, 12.58 and 13.21% in the total counts following application of 0.4, 0.6 and 0.8% methoprene, respectively, which was insignificant at 5% level compared to control. The highest concentration (1.0%) caused an appreciable damage to haemocytes and reduced their population by 34.35% compared to control.

After 1 day

The THCs of untreated and acetone treated (control) 6th instar larvae of corresponding age were found to be 41710 ± 3682.44 and 41000 ± 3162.63 cells/mm^3 of haemolymph. The lowest concentration induced a slight increase in THC that, however, was insignificant at 5% level. The other three concentrations viz 0.6, 0.8 and 1.0% caused reduction of 11.76%, 24.17% and 31.66% in comparison to control.

After 3 days

The THCs of untreated and control larvae were found to be 71050 ± 7871.58 and 72610 ± 6956.25 cells/mm^3 of haemolymph. The higher concentrations viz 0.6, 0.8 and 1.0% methoprene, respectively, caused a reduction of 16.54%, 31.44% and 43.70% compared to control.

After 5 days

Five days following the above-mentioned serial concentrations except the lowest one of methoprene, the total counts were reduced by 14.86, 28.37, and 32.59% compared to control. The control and untreated larvae contained 58090 ± 2376.31 and 58970 ± 4091.17 cells/mm^3 of haemolymph, respectively.

After pupal ecdysis

The total counts of individual pupae varied greatly in the untreated as well as treated groups thereby showing high standard error values. The THCs of untreated and control pupae respectively was 11130 ± 2063.35 and 10410 ± 1538.94 cells/mm^3 of haemolymph. The two lower concentrations (0.4 and 0.6%) of methoprene reduced the THCs by 6.34 and 15.37% respectively in comparison to control. The alterations in THCs were statistically insignificant.

5.3 DHCs of 6th instar larvae and pupae of *Diacrisia obliqua* affected with different concentrations of Methoprene.

Besides pathology and total haemocyte counts, the DHCs of treated 6th instar larvae were also determined after 6 hrs, 1 day, 3 days and 5 days following treatment of 2 day old 6th instar larvae with aforesaid concentrations of methoprene. When the treated 6th instar larvae moulted to pupal stage, the DHCs were taken in one-day-old pupae. The account of DHC at various time intervals is given below

After 6 hrs (Table-61, Fig. 35)

Prohaemocytes and plasmatocytes were slightly reduced with respect to increasing concentration of methoprene but the reduction was insignificant at 5% level of significance. However, granulocytes showed a linear increase from 3.12% in control to 3.92, 4.78 5.68 and 7.82%, respectively, following 0.4, 0.6, 0.8 and 1.0%

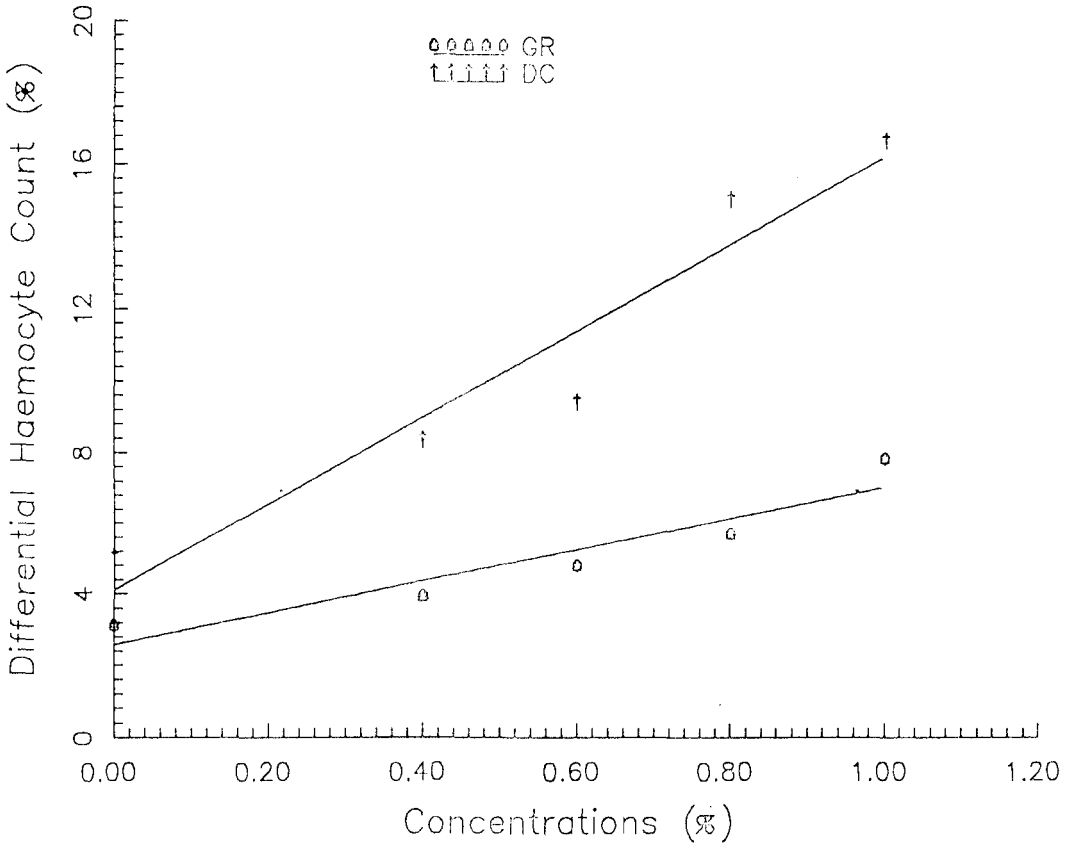

Fig. 35 : Correlation between the Differential Haemocyte Count (%) and various concentrations of methoprene after 6 hrs of the treatment on one day old 6th instar larvae of Pieris isia obliqua.

methoprene (Y = 2.58 + 4.43 X, r = 0.9395, P<0.001). The highest concentration (1.0%) of methoprene showed a significant increase at 5% level (t= 2.939, P<0.05). The relative populations of spherulocytes, oenocytoids and coagulocytes were insignificantly affected at all concentrations.

Furthermore, the disintegrating and unidentified haemocyte percentage was linearly increased 6 hrs following increasing concentrations (Y= 4.11 + 12.15 X, r = 0.9627, P<0.001) which was significant with 0.8% (15.06%, t=3.963, P<0.05) and 1.0% (16.72%, t=3.459, P<0.05) methoprene, respectively, compared to control (5.04%).

After 1 day (Table-62, Fig. 36)

After 1 day of treatment, the prohaemocytes showed linear increase (Y = 34.72 + 8.86 X, r = 0.9592, P<0.001) which, however, was statistically insignificant. On the other hand, plasmatocytes were significantly reduced to 36.86% by 0.6% (t= 2.948, P<0.05) and 26.74% by 1.0% methoprene (t= 4.017, P<0.05). The regression between plasmatocyte population and concentration strength of methoprene yielded a negative linear correlation coefficient (Y= 51.13 - 22.18 X, r = - 0.9934, P<0.001). Granulocytes showed an insignificant increase with respect to 0.4 and 0.6% methoprene, however, on increasing the concentration to 0.8 and 1.0% there was slight reduction in their population, which nevertheless was insignificant statistically at 5% level. Compared to that of control, spherulocytes could not be identified in all treated smears. Oenocytoids, although showed an increase in the relative population, it however was not significant. Coagulocytes were significantly increased to 3.88% (t= 3.01, p<0.05) following the highest concentration as compared to control population (2.16%). Unidentified and disintegrating haemocytes were significantly high at 0.8% (15.88%, t= 3.307, P<0.05) and 1.0% (20.30%,t=4.47, p<0.05) compared to control (7.46%), and yielded a positive linear correlation with increasing concentration of methoprene (Y= 5.53 + 13.11 X, r = 0.9376, P<0.001)

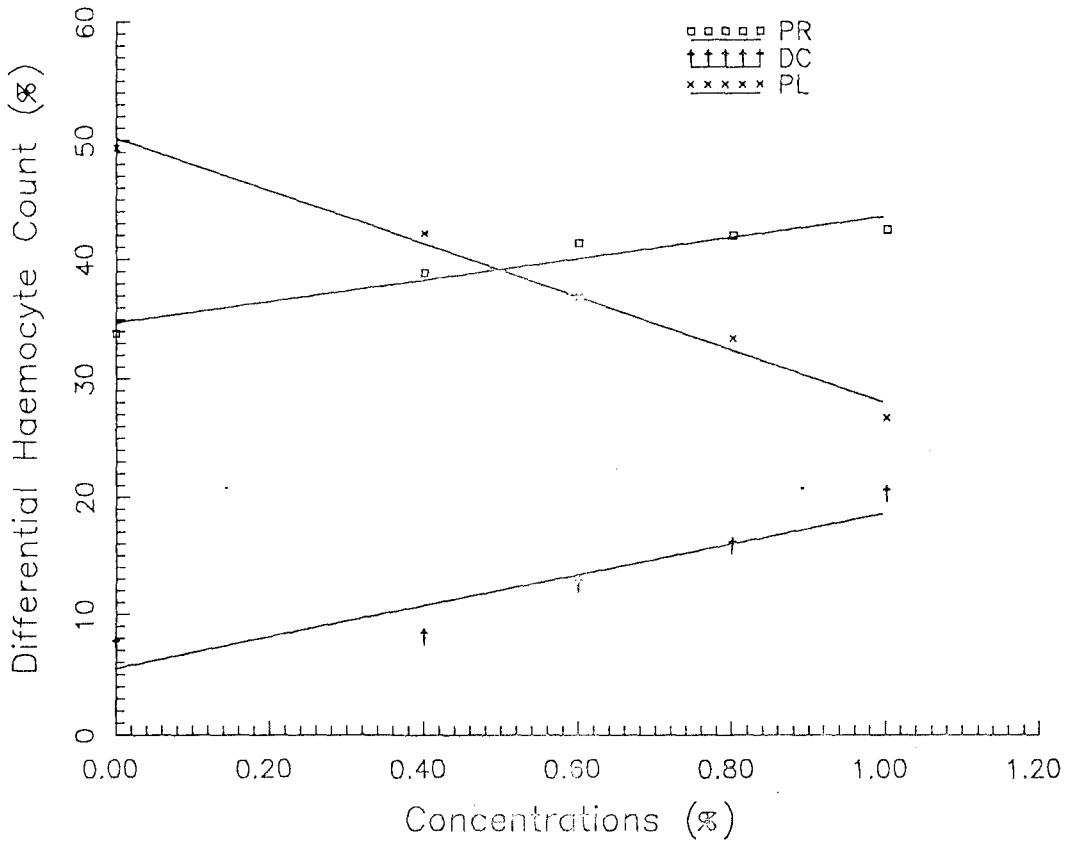

Fig. 36 : Correlation between the Differential Haemocyte Count (%) and various concentrations of methoprene after one day of the treatment on one day old 6th instar larvae of Diacrisia obliqua.

After 3 days (Table-63, Fig. 37)

Three day following treatment with various concentrations, the prohaemocytes and granulocytes population exhibited a concentration based increase, it, however, was insignificant ($P>0.05$) at all applied concentrations. Plasmatocytes showed a reduction from 54.34% in control to 46.48% ($t= 2.229$, $P>0.05$), 43.32% ($t =2.656$, $P>0.05$), 35.00% ($t= 3.462$ $P<0.05$) and 31.56% ($t=4.689$, $P<0.05$) following treatment with 0.4, 0.6, 0.8 and 1.0% methoprene, respectively. Consequently, the regression between concentration strength and plasmatocyte percentage yielded a negative linear correlation ($y= 55.24 - 23.39 X$, $r = - 0.9878$, $P<0.001$). Spherulocytes and coagulocytes populations showed a reduction in blood smears of treated insects as compared to control and were altogether absent in the smears affected with the highest concentration. The unidentified and disintegrating haemocyte population showed a positive linear correlation with respect to increasing concentrations of methoprene ($Y= 4.38 + 14.95 X$, $r = 0.9434$, $P<0.001$). The enhancement in the population was significant by 0.8 and 1.0% methoprene ($t=3.764$ and $t= 3.700$ respectively, $P<0.05$).

After 5 days (Table-64, Fig. 38)

When the smears of treated larvae were observed after 5 days of application with various concentrations of methoprene, the prohaemocytes population showed a steady increase with respect to serial concentrations and regression between the two yielded a positive linear correlation ($Y= 14.67 + 26.66 X$, $r = 0.9075$, $P<0.001$). The rise in population was significant by 0.8% (40.28%, $t=3.695$, $P<0.05$) and 1.0% methoprene (43.04%, $t=4.018$, $P<0.05$) in comparison to the control (18.82%). On the other hand, plasmatocytes were reduced from 53.86% in control to 50.58% ($t=1.362$, $P>0.05$), 46.34% ($t=2.053$, $P>0.05$), 30.42% ($t= 3.708$, $P<0.05$) and 30.64% ($t=3.425$, $P<0.05$), respectively, following treatment with 0.4, 0.6, 0.8 and 1.0% methoprene. The fall in relative percentage was significant by two higher concentrations. The regression between concentration strength and plasmatocyte percentage yielded a negative linear correlation ($Y= 57.14 - 26.38 X$, $r = - 0.9118$, $P<0.001$). Likewise, granulocyte population, too, showed a negative linear correlation with increasing concentrations of

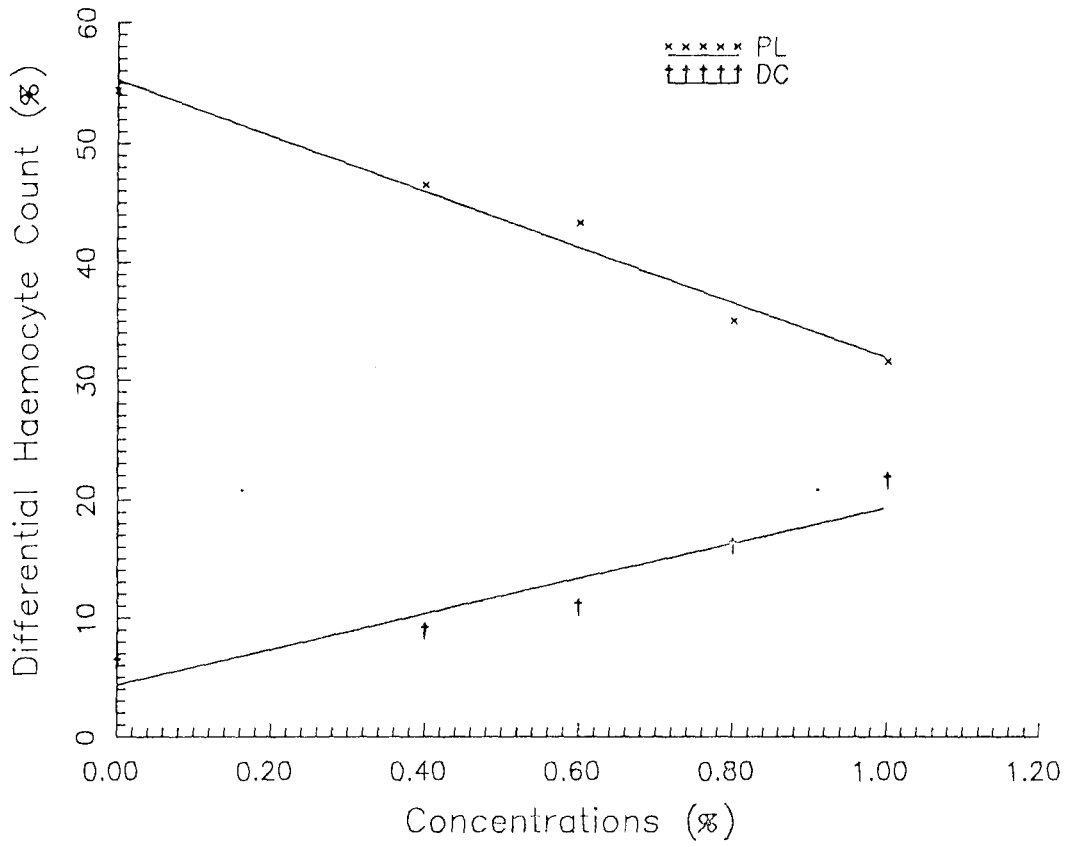

Fig. 37 : Correlation between the Differential Haemocyte Count (%) and various concentrations of methoprene after 3 days of the treatment on one day old 6th instar larvae of Diacrisia obliqua.

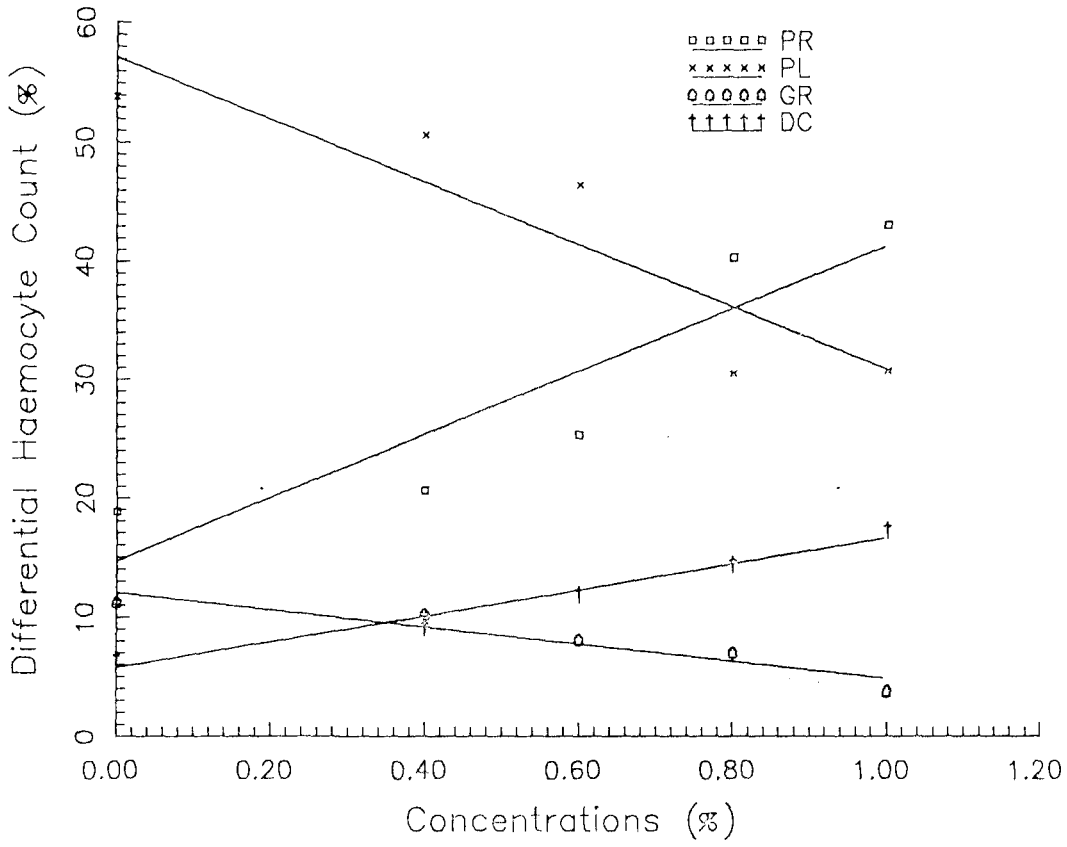

Fig. 38 : Correlation between the Differential Haemocyte Count (%) and various concentrations of methoprene after 5 days of the treatment on one day old 6th instar larvae of Diacrisia obliqua.

methoprene (Y= 12.07 - 07.25 X, r = - 0.9480, P<0.001). The reduction was significant at the highest concentration (t=4.009, P<0.05). Spherulocyte, oenocytoid and coagulocyte populations although underwent alterations in the treated larvae, but they were insignificant at 5% level. The percentage of unidentified and disintegrating cells was significantly high at 0.6, 0.8 and 1.0% methoprene (11.94%, t= 2.789; 14.34%, t = 3.237; 17.20%, t= 4.241, respectively) compared to that of control (6.48%). The regression between concentration strength and population of damaged cells yielded a positive linear correlation (Y= 5.78 + 10.0 X, r = 0.9867, P<0.001).

After pupal ecdysis (Table-65)

Prohaemocytes and granulocytes were significantly less in the pupae affected with 0.4 and 0.6% methoprene. Whereas, damaged cells showed a significant increase in population at the above mentioned concentrations.

III. Ultrastructure of abnormal haemocytes

Treatment of 6th instar larvae of *D. obliqua* with various concentrations of different selected insecticides (acephate, aminocarb and cypermethrin) and hormone analogues (muristerone and methoprene) induced abnormalities in the structure of various types of haemocytes described before. Although these insecticides and hormones belong to different chemical groups, the histopathological symptoms induced in the haemocytes subsequent to the treatment of the larvae were of almost similar nature, characterized by the cytoplasmic and nuclear deformities. However, the degree and extent of the damage was dependent on the concentration of chemicals applied.

Observation under the light microscope revealed that the effects of various chemicals on the haemocytes were slight, moderate and intense, which have already been described in detail under the relevant headings. The histopathological symptoms in various types of haemocytes were also studied at the ultrastructural level to get a better understanding of cellular damage. Since the kinds of abnormalities in various

haemocytes under the influence of selected insecticides and hormones were same at the ultrastructural level also, given below, is an account of the general pathological symptoms encountered in various haemocyte types irrespective of the chemical applied.

Prohaemocytes

Prohaemocytes showed slight or moderate changes in structure in response to the application of insecticides. However, sometimes, disintegrating prohaemocytes were also encountered. The nuclei of slightly affected prohaemocytes showed only mild irregularities. However, several outgrowths resembling long tentacles also appeared on the cell surface (Plate XXII, Fig. E; Plate-XXIII, Fig. A & D). The cell organelles were occasionally present. Since, the cytoplasm was scanty, only a few cytoplasmic organelle viz., endoplasmic reticulum, mitochondria and lytic vacuoles were present. Plate-XXII, Fig. D shows a prohaemocyte with large eccentric nucleus consisting of less distinct heterochromatin and euchromatin. The cytoplasm contained numerous lysed organelles. Highly affected prohaemocytes showed severe damage. The cells took on a ragged aspect as the degeneration proceeded and the cytoplasm as well as nucleus appeared irregularly frayed and torn (Plate-XXII, Fig. A, B & F). The cell membrane and nuclear membrane were completely destroyed at certain places and from the gaps, consequently formed, outflow of cytoplasmic and nuclear contents took place. A small fragment of nucleus, containing heterochromatin and euchromatin was also visible in the cytoplasm (Plate-XXII, Fig. C).

Plasmatocytes

Plasmatocytes exhibited all kinds of cytoplasmic and nuclear abnormalities in response to the application of various insecticides. The initial pathological symptoms were characterized by irregular cell boundaries due to formation of pseudopodia like structures as well as small processes accompanied by few vacuoles in the cytoplasm. Large networks of rough endoplasmic reticulum were also observed (Plate-XXIII, Fig. G; Plate-XXV, Fig. B, C & D). This was followed by more intense vacuolization represented by the appearance of numerous small as well as large vacuoles in the

cytoplasm (Plate-XXIII, Fig. B, E & H; Plate-XXV, Fig. E). The cytoplasmic organelles also became less distinct. The nuclear envelope was found broken at places and discharge of nuclear material into the cytoplasm took place. Some plasmatocytes became more electron dense. Consequently, the distinction between nucleus and cytoplasm was lost (Plate-XXIII, Fig. B, E & H). In some other plasmatocytes, in addition to the appearance of small vacuoles in the cytoplasm, the nuclear membrane was disrupted at many places and the nuclear contents seemed to be mixed with that of the cytoplasm (Plate-XXV, Fig. A). The mitochondria in one of these plasmatocytes was hypertrophied (Plate-XXIV, Fig. E). The plasmatocytes that were highly affected underwent disintegration (Plate-XXIV, Fig. D & F) which, was characterized by distortion in cell shape, irregular cell surface and indistinguishable cell organelles. The clear distinction between nucleus and cytoplasm, as present in normal cells, was lost to some extent. The nuclear contents also became indistinguishable.

Numerous large tentacle-like processes were found to be arising from the surface of plasmatocytes. Some processes showed coalescence with each other resulting in the formation of vacuole like spaces (Plate-XXIV, Fig. A). Plate-XXIV, Fig. A shows a plasmatocyte with distorted shape, long extension on one end, and the presence of well rounded fragments in the cytoplasm which were composed of the substances resembling the nuclear material. The cell membrane was pushed outwards and one of the spheres was protruded from there (double arrow). The cytoplasmic extensions at the spindle end were also present in some other plasmatocytes (Plate-XXIII, Fig. C; Plate-XXIV, Fig. B).

Some plasmatocytes (Plate-XXVI, Fig. A, B & C) showed the presence of a small nucleus like fragment comprised of chromatin material and bounded by a double membrane in the vicinity of a larger regular nucleus which was also bounded by a double membrane and contained distinct heterochromatin and euchromatin material. The cytoplasm of these plasmatocytes sometimes contained medium sized vacuoles, moreover, among the organelles mitochondria and free ribosomes were the most distinct. Another severely affected plasmatocyte (Plate-XXIII, Fig. F) exhibited disintegration of cytoplasmic structure and loss of cell organelles. The cell gave the indication of being stretched. Furthermore, along the margins of cells as well as

nucleus long thread like wavy membranous layers were present. The nucleus appeared swollen and stretched into another lobe.

Granulocytes

The haemolymph of *Diacrisia obliqua* larvae was found to contain relatively small population of granulocytes compared to plasmatocytes and prohaemocytes, as also confirmed by the differential haemocyte counts at various stages under normal as well as treated conditions. The less affected granulocytes exhibited occasional vacuolization in cytoplasm (Plate-XXVII, Fig. B), ragged appearance (Plate-XXVI, Fig. F; Plate- XXVII, Fig. A, B & D), phagocytic vacuoles and indistinct cytoplasmic organelles. Nucleus was swollen in many cases (Plate-XXVI, Fig. G) and became shrinked (Plate-XXVII, Fig. A & D) and fragmented in others (Plate-XXVII, Fig. A & B). The round or ovoid shape was generally retained. Granulocytes affected with higher concentrations of insecticides generally became unrecognizable, however, few cells which could be identified, though sometimes with a little ambiguity, displayed a definitely ragged appearance. The plasma membrane became discontinuous at many places, thereby giving the cells an irregular surface (Plate-XXVI, Fig. D; Plate-XXVII, Fig. E). Sometimes, granules were also discharged from the cytoplasm (Plate-XXVII, Fig. E). Occasionally, lysis of cell organelles was occurred (Plate-XXVI, Fig. D; Plate-XXVII, Fig. C & E). Nucleus was intact in some granulocytes (Plate XXVII, Fig. E) containing distinct heterochromatin and euchromatin, but in others, the nucleus was either absent (Plate-XXVI, Fig. D) or became highly electron dense with indistinct heterochromatin and euchromatin (Plate-XXVII, Fig. E). Plate-XXVI, Fig. D shows completely distorted shape of granulocytes.

Oenocytoids

Like light microscopic study, the ultrastructure of the oenocytoids affected with various concentrations of insecticides and hormones did not reveal any marked abnormality in either cytoplasm or nucleus. The cytoplasmic matrix was homogeneous containing numerous free ribosomes and microtubules. A few mitochondria one or two small granules and rarely one or two vacuoles represented the cytoplasmic organelles.

The nucleus was eccentric and small consisting of distinct heterochromatin and euchromatin surrounded by a double membrane. In some oenocytoids nucleus appeared somewhat swollen (Plate-XXVIII, Fig. A). In other oenocytoids the nucleus became irregular in shape and next to it, a small fragment of nucleus was also present. The cytoplasm contained homogeneous ground matrix composed of abundant free ribosomes, microtubules and a few mitochondria. In addition to these, numerous moderate sized granules also appeared in the cytoplasm (Plate-XXVIII, Fig. B). The highly affected oenocytoids displayed some irregularity in the cell membrane, appearance of a few tentacle like processes and ragged cytoplasmic structure. Numerous, very minute vacuoles also appeared in the cytoplasm. The nuclear envelope was disrupted at several places and the nuclear contents were mingled with the cytoplasmic contents (Plate-XXVIII, Fig. C).

Disintegrating and unidentifiable haemocytes

The general haematological changes, subsequent to the treatment, were recognized as progressive stages that were designated as slight, moderate and intense. In the first two stages, haemocytes usually did not lose their identity, whereas, the intense stage was characterized by cell agglutination, considerable distortion and subsequent disintegration. The haemocytes undergoing various phases of disintegration could not be unambiguously identified into definite types. The severe haematological deformities, as observed under light microscope, have already been discussed in detail under the relevant headings. These abnormalities in the affected haemocytes were also confirmed ultrastructurally under transmission electron microscope.

The affected haemocytes showed extensive vacuolization (Plate-XXIX, Fig. A & B) and in some cases the vacuolization was so intense that the entire cytoplasm exhibited a network of vacuoles. The cytoplasmic organelles viz. mitochondria, endoplasmic reticulum, Golgi bodies, lysosomes etc., became indistinguishable (Plate-XXVIII, Fig. D, E & F). In spite of excessive cytoplasmic vacuolization, the nucleus of some affected haemocytes did not lose its integrity (Plate-XXVIII, Fig. D; Plate-XXIX, Fig. A & B). In some haemocytes vacuoles did not appear, however, the cytoplasm

was ragged and the nucleus was not distinct. The cytoplasm exhibited presence of small granules. Mitochondria, endoplasmic reticulum and numerous free ribosomes were distinct (Plate-XXIX, Fig. C & D).

Plate-XXIX, Fig. E shows a haemocyte of questionable identity, exhibiting long tentacle like processes emerging from the surface. The cytoplasmic organelles are indistinct and the fragments visible in the picture presumably represent remains of the nucleus. The extracellular space in the immediate vicinity of the normal as well as lysing haemocytes generally contained finely granular particulate material believed to be coagulated haemolymph. In various sections of haemolymph, affected with the higher concentrations of insecticides, some dark coloured particulate matter, slightly coarser than the coagulated haemolymph, was also evident which, presumably, was disintegrated chromatin material (arrow). Some haemocytes showed lysis of cytoplasmic organelles, although a few large granules, rather spherules were present (Plate-XXIX, Fig. F; Plate-XXX- Fig. A). These haemocytes had a resemblance with oenocytoids. Some haemocytes showed fragmented nucleus which appeared as if fractured into two parts. The cytoplasm of these haemocytes showed very large granules almost completely filling the cytoplasm (Plate-XXX, Fig. B). Various small vacuoles were also present in the cytoplasm. The cytoplasm of another haemocyte (Plate-XXX, Fig. C) was completely filled with two large granules, one containing light coloured granular substance, whereas, the other was electron dense. The nucleus appeared hidden and only a small part of it was visible near the periphery of the cell. Plate-XXX, Fig. D shows a haemocyte containing two large electron dense structures in the cytoplasm. One phagocytic vacuole was also present. A part of nucleus was also evident with distinct heterochromatin and euchromatin material. Still another highly affected haemocyte (Plate-XXX, Fig. E) showed excessively disrupted cytoplasmic structure consisting of clumps of some electron dense material and numerous small granules. The nucleus was distinct with distinguishable heterochromatin and euchromatin. Other cell organelles became indistinguishable. The cell surface showed many large tentacles like outgrowths.

Table 36: Total Haemocyte Counts determined at various time intervals following the topical application of various concentrations of Acephate on 1-2 day old 6th instar larvae of *Diacrisia obliqua*

Concentrations (%) 2 µl / larva	Time Interval				
	Six Hours (Mean ± S.E.)	One Day (Mean ± S.E.)	Three Day (Mean ± S.E.)	Five Day (Mean ± S.E.)	Pupa (Mean ± S.E.)
Untreated	35590.0 ± 2504.92	41560.0 ± 3532.40	72830.0 ± 3896.17	60410.0 ± 3822.15	9060.0 ± 1903.24
Solvent Treated	34310.0 ± 2998.97	39180.0 ± 3937.62	73000.0 ± 4752.39	63800.0 ± 6787.07	9600.0 ± 1334.26
0.04	41780.0 ± 6892.58	49300.0 ± 7086.11	67970.0 ± 7311.51	68070.0 ± 4085.82	12930.0 ± 2260.01
0.08	45720.0 ± 4332.57	46450.0 ± 3307.68	69930.0 ± 6892.98	52900.0 ± 7457.94	11980.0 ± 1959.56
0.1	27360.0 ± 7666.35	26720.0 ± 2787.05	52320.0 ± 6582.07	38170.0 ± 9725.49	8010.0 ± 1988.87
0.2	14730.0 ± 2396.07	8940.0 ± 1392.43	28910.0 ± 4106.14	22760.0 ± 6456.50	--

Table 37: Total Haemocyte Counts determined at various time intervals following the topical application of various concentrations of Aminocarb on 1-2 day old 6th instar larvae of *Diacrisia obliqua*

Concentrations (%) 2 µl / larva	Time Interval				
	Six Hours (Mean ± S.E.)	One Day (Mean ± S.E.)	Three Day (Mean ± S.E.)	Five Day (Mean ± S.E.)	Pupa (Mean ± S.E.)
Untreated	36790.0 ± 3137.50	38720.0 ± 6309.73	72650.0 ± 9816.37	63180.0 ± 8094.27	11160.0 ± 3096.93
Solvent Treated	36860.0 ± 4534.93	40830.0 ± 5662.23	71000.0 ± 8100.95	65400.0 ± 6620.37	10880.0 ± 1992.52
0.1	46450.0 ± 6432.24	49290.0 ± 5998.03	77110.0 ± 9215.05	59740.0 ± 2794.03	12480.0 ± 2503.43
0.2	29210.0 ± 3047.22	30970.0 ± 4326.51	60260.0 ± 5936.65	53050.0 ± 2523.69	8650.0 ± 2446.43
0.4	24690.0 ± 1945.98	26440.0 ± 2562.49	49660.0 ± 7012.19	32800.0 ± 4645.51	7210.0 ± 1491.59
0.6	13180.0 ± 2879.78	8550.0 ± 2399.84	21840.0 ± 4579.86	20480.0 ± 3433.28	--

Table 38: Total Haemocyte Counts determined at various time intervals following the topical application of various concentrations of Cypermethrin on 1-2 day old 6th instar larvae of *Diacrisia obliqua*

Concentrations (%) 2 µl / larva	Time Interval				
	Six Hours (Mean ± S.E.)	One Day (Mean ± S.E.)	Three Day (Mean ± S.E.)	Five Day (Mean ± S.E.)	Pupa (Mean ± S.E.)
Untreated	38320.0 ± 4919.52	44300.0 ± 7695.13	66590.0 ± 10100.8	61850.0 ± 5589.57	9440.0 ± 1582.59
Solvent Treated	38520.0 ± 3691.43	42680.0 ± 6226.41	68850.0 ± 8700.82	59980.0 ± 3684.79	10860.0 ± 3029.99
0.0025	44580.0 ± 8751.62	59680.0 ± 8872.66	75440.0 ± 7406.25	64730.0 ± 6599.44	12060.0 ± 4979.32
0.005	46290.0 ± 6951.03	63730.0 ± 7341.91	78490.0 ± 6496.68	52720.0 ± 3287.27	8900.0 ± 2179.28
0.01	22370.0 ± 2601.37	27400.0 ± 4522.83	35840.0 ± 5179.95	27770.0 ± 3242.25	
0.015	11240.0 ± 4972.40	9750.0 ± 4688.28	9840.0 ± 3715.49	10800.0 ± 4620.42	--

Table 39: Total Haemocyte Counts determined at various time intervals following the topical application of various concentrations of Muristerone on 1-2 day old 6th instar larvae of *Diacrisia obliqua*

Concentrations (%) 2 µl / larva	Time Interval				
	Six Hours (Mean ± S.E.)	One Day (Mean ± S.E.)	Three Day (Mean ± S.E.)	Five Day (Mean ± S.E.)	Pupa (Mean ± S.E.)
Untreated	32010.0 ± 4559.86	42500.0 ± 7494.46	69070.0 ± 4332.28	50260.0 ± 2149.85	10820.0 ± 1519.01
Solvent Treated	33390.0 ± 4113.57	41080.0 ± 2323.20	70320.0 ± 5427.33	51370.0 ± 1327.93	10970.0 ± 1815.74
0.5	36690.0 ± 4900.37	48300.0 ± 7058.91	84950.0 ± 7148.72	56020.0 ± 6019.25	13250.0 ± 1895.59
1.0	39520.0 ± 7021.94	51260.0 ± 6116.30	87070.0 ± 7148.72	65840.0 ± 5667.50	15210.0 ± 2805.10
1.5	42670.0 ± 4207.04	54590.0 ± 4335.30	60270.0 ± 7596.69	45470.0 ± 4466.17	--
2.0	25120.0 ± 4232.95	32860.0 ± 6305.54	44510.0 ± 2406.31	--	--

Table 40: Total Haemocyte Counts determined at various time intervals following the topical application of various concentrations of Methoprene on 1-2 day old 6th instar larvae of *Diacrisia obliqua*

Concentrations (%) 2 µl / larva	Time Interval				
	Six Hours (Mean ± S.E.)	One Day (Mean ± S.E.)	Three Day (Mean ± S.E.)	Five Day (Mean ± S.E.)	Pupa (Mean ± S.E.)
Untreated	30870.0 ± 2989.38	41710.0 ± 3682.44	71050.0 ± 7871.58	58970.0 ± 4091.17	11130.0 ± 2063.35
Solvent Treated	31870.0 ± 3454.73	41000.0 ± 3162.63	72610.0 ± 6956.25	58090.0 ± 2376.31	10410.0 ± 1538.94
0.4	33890.0 ± 3059.31	43980.0 ± 8477.42	78690.0 ± 4597.21	62210.0 ± 7021.01	9750.0 ± 1313.49
0.6	35880.0 ± 3050.41	36180.0 ± 3433.42	60600.0 ± 4946.59	49460.0 ± 6034.74	8810.0 ± 937.67
0.8	36080.0 ± 5672.18	31090.0 ± 3655.11	49780.0 ± 6102.00	41610.0 ± 4452.65	
1.0	20860.0 ± 4767.24	28020.0 ± 5483.86	40880.0 ± 7171.70	39160.0 ± 7883.06	--

Table 41: Differential Haemocyte Counts determined after 6 hrs. following the topical application of various concentrations of Acephate on 1-2 day old 6th instar larvae of *Diacrisia obliqua*

Concentrations (%) 2 µl/larva	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Granulocytes % ± S. E.	Spherulocytes % ± S. E.	Oenocytoids % ± S. E.	Coagulocytes % ± S. E.	Disintegrating Cells % ± S. E.
Untreated	37.06 ± 2.09	43.08 ± 2.85	04.38 ± 0.61	02.20 ± 0.30	01.60 ± 0.39	00.92 ± 0.41	10.88 ± 2.41
Solvent Treated	38.36 ± 2.43	42.10 ± 2.94	04.12 ± 0.44	01.99 ± 0.45	02.02 ± 0.43	01.02 ± 0.53	10.36 ± 0.69
0.04	40.38 ± 4.59	45.30 ± 2.46	00.40 ± 0.25	00.30 ± 0.19	01.04 ± 0.28	01.06 ± 0.51	11.26 ± 2.90
0.08	48.34 ± 2.01	32.96 ± 1.01	02.28 ± 0.56	01.14 ± 0.52	0.74 ± 0.50	02.18 ± 0.53	12.48 ± 1.20
0.1	41.40 ± 3.39	26.88 ± 2.30	06.04 ± 1.41	03.08 ± 0.90	03.54 ± 0.90	03.08 ± 0.34	16.00 ± 1.79
0.2	46.30 ± 2.86	07.96 ± 0.84	03.20 ± 0.91	00.00	09.29 ± 1.47	05.08 ± 0.88	28.16 ± 3.05

Table 42: Differential Haemocyte Counts determined after 1 day following the topical application of various concentrations of Acephate on 1-2 day old 6th instar larvae of *Diacrisia obliqua*

Concentrations (%) 2 µl/larva	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Granulocytes % ± S. E.	Spherulocytes % ± S. E.	Oenocytoids % ± S. E.	Coagulocytes % ± S. E.	Disintegrating Cells % ± S. E.
Untreated	31.30 ± 3.50	49.14 ± 4.12	05.56 ± 1.46	02.30 ± 0.41	02.98 ± 0.61	02.06 ± 0.23	06.68 ± 1.05
Solvent Treated	32.50 ± 0.89	48.88 ± 1.57	05.16 ± 0.50	03.06 ± 0.34	02.92 ± 0.87	01.88 ± 0.60	05.48 ± 0.40
0.04	38.18 ± 2.45	33.94 ± 3.12	10.52 ± 1.51	02.36 ± 0.60	01.88 ± 0.58	02.34 ± 0.71	10.86 ± 1.27
0.08	44.68 ± 3.04	31.30 ± 2.16	00.00	00.42 ± 0.26	05.00 ± 0.47	00.66 ± 0.43	17.94 ± 0.63
0.1	26.08 ± 2.06	29.29 ± 4.37	01.18 ± 0.43	00.40 ± 0.26	05.60 ± 0.85	02.96 ± 0.67	34.56 ± 3.46
0.2	21.34 ± 2.01	22.58 ± 2.91	02.22 ± 0.46	00.00	10.22 ± 0.84	03.58 ± 0.93	40.08 ± 4.17

Table 43: Differential Haemocyte Counts determined after 3 days following the topical application of various concentrations of Acephate on 1-2 day old 6th instar larvae of *Diacrisia obliqua*

Concentrations (%) 2 µl/larva	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Granulocytes % ± S. E.	Spherulocytes % ± S. E.	Oenocytoids % ± S. E.	Coagulocytes % ± S. E.	Disintegrating Cells % ± S. E.
Untreated	24.22 ± 0.66	53.94 ± 3.84	07.76 ± 0.81	03.36 ± 0.54	02.70 ± 0.50	02.48 ± 0.86	05.54 ± 1.19
Solvent Treated	27.94 ± 2.08	52.92 ± 2.04	07.76 ± 0.47	03.16 ± 0.34	00.96 ± 0.39	01.92 ± 0.61	05.34 ± 0.58
0.04	26.36 ± 1.72	50.08 ± 1.36	08.72 ± 1.61	01.86 ± 0.44	01.76 ± 0.20	01.42 ± 0.42	09.78 ± 1.49
0.08	31.84 ± 2.43	40.55 ± 2.46	05.96 ± 1.25	00.00	04.92 ± 0.68	03.40 ± 0.70	12.82 ± 0.92
0.1	39.30 ± 2.70	22.12 ± 4.86	00.00	00.00	09.02 ± 1.36	04.94 ± 0.68	24.62 ± 2.16
0.2	31.00 ± 2.44	14.98 ± 2.59	00.00	00.00	13.90 ± 1.55	06.00 ± 1.19	34.14 ± 2.65

Table 44: Differential Haemocyte Counts determined after 5 days following the topical application of various concentrations of Acephate on 1-2 day old 6th instar larvae of *Diacrisia obliqua*

Concentrations (%) 2 µl/larva	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Granulocytes % ± S. E.	Spherulocytes % ± S. E.	Oenocytoids % ± S. E.	Coagulocytes % ± S. E.	Disintegrating Cells % ± S. E.
Untreated	24.60 ± 1.86	45.18 ± 3.27	12.90 ± 2.20	04.78 ± 0.92	02.12 ± 0.36	02.92 ± 0.43	07.54 ± 1.11
Solvent Treated	21.72 ± 2.66	44.32 ± 4.05	13.74 ± 1.99	04.20 ± 0.67	03.04 ± 0.40	02.20 ± 0.38	07.06 ± 0.65
0.04	27.46 ± 2.11	37.76 ± 2.69	15.70 ± 0.62	03.26 ± 0.82	03.24 ± 0.50	02.02 ± 0.42	10.56 ± 1.17
0.08	30.44 ± 1.60	32.24 ± 3.44	10.46 ± 0.91	00.00	04.88 ± 1.00	03.96 ± 0.66	18.06 ± 1.39
0.1	30.32 ± 3.53	25.62 ± 2.43	01.42 ± 0.59	00.00	05.16 ± 0.65	04.78 ± 0.81	32.70 ± 2.75
0.2	29.84 ± 1.06	13.70 ± 1.46	00.00	00.00	06.78 ± 1.18	07.40 ± 0.99	42.28 ± 1.85

Table 45: Differential Haemocyte Counts determined in pupa following the topical application of various concentrations of Acephate on 1-2 day old 6th instar larvae of *Diacrisia obliqua*

Concentrations (%) 2 µl/larva	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Granulocytes % ± S. E.	Spherulocytes % ± S. E.	Oenocytoids % ± S. E.	Coagulocytes % ± S. E.	Disintegrating Cells % ± S. E.
Untreated	16.46 ± 2.24	69.16 ± 1.22	02.24 ± 0.62	00.00	00.00	03.54 ± 0.66	08.62 ± 1.38
Solvent Treated	17.72 ± 2.49	67.00 ± 3.12	02.18 ± 0.63	00.00	00.00	02.60 ± 0.88	10.56 ± 0.98
0.04	13.16 ± 0.94	71.18 ± 1.89	01.48 ± 0.71	00.00	00.00	03.72 ± 1.08	10.46 ± 0.90
0.08	08.58 ± 1.21	72.62 ± 2.07	00.00	00.00	00.00	02.10 ± 0.71	16.70 ± 2.23
0.1	10.34 ± 1.00	68.44 ± 2.79	00.00	00.00	00.00	00.00	21.24 ± 1.42
0.2	00.00	00.00	00.00	00.00	00.00	00.00	00.00

Table 46: Differential Haemocyte Counts determined after 6 hrs. following the topical application of various concentrations of Aminocarb on 1-2 day old 6th instar larvae of *Diacrisia obliqua*

Concentrations (%) 2 µl/larva	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Granulocytes % ± S. E.	Spherulocytes % ± S. E.	Oenocytoids % ± S. E.	Coagulocytes % ± S. E.	Disintegrating Cells % ± S. E.
Untreated	38.36 ± 2.81	48.46 ± 2.97	03.08 ± 0.95	01.72 ± 0.53	01.00 ± 0.27	01.42 ± 0.41	07.36 ± 1.35
Solvent Treated	37.30 ± 3.20	47.90 ± 1.88	04.88 ± 0.77	02.02 ± 0.59	01.32 ± 0.44	00.54 ± 0.34	06.04 ± 0.70
0.1	34.84 ± 2.70	51.00 ± 2.14	04.78 ± 0.43	00.62 ± 0.29	01.08 ± 0.49	00.80 ± 0.57	06.88 ± 1.06
0.2	19.04 ± 0.93	58.52 ± 3.01	04.04 ± 0.85	00.48 ± 0.30	01.62 ± 0.58	01.94 ± 0.36	14.38 ± 1.99
0.4	26.22 ± 2.43	37.74 ± 2.27	01.94 ± 0.60	00.00	03.44 ± 0.64	01.48 ± 0.77	29.20 ± 1.79
0.6	33.18 ± 8.74	20.86 ± 2.47	01.02 ± 0.46	00.00	09.28 ± 1.90	00.00	35.82 ± 5.43

Table 47: Differential Haemocyte Counts determined after 24 hrs. following the topical application of various concentrations of Aminocarb on 1-2 day old 6th instar larvae of *Diacrisia obliqua*

Concentrations (%) 2 µl/larva	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Granulocytes % ± S. E.	Spherulocytes % ± S. E.	Oenocytoids % ± S. E.	Coagulocytes % ± S. E.	Disintegrating Cells % ± S. E.
Untreated	32.32 ± 2.60	50.98 ± 1.55	07.00 ± 1.86	01.14 ± 0.55	01.82 ± 0.63	02.26 ± 0.46	04.48 ± 0.82
Solvent Treated	33.78 ± 1.57	48.46 ± 3.14	06.24 ± 1.16	02.14 ± 0.77	02.18 ± 0.95	01.56 ± 0.42	05.68 ± 1.19
0.1	38.98 ± 3.85	47.00 ± 2.55	01.36 ± 0.43	01.93 ± 0.62	01.06 ± 0.30	00.00	09.62 ± 1.05
0.2	39.66 ± 5.09	37.50 ± 4.74	00.00	01.22 ± 0.75	02.44 ± 0.51	00.00	19.06 ± 1.93
0.4	38.62 ± 2.31	25.28 ± 3.45	00.00	000.00	04.16 ± 0.51	01.60 ± 0.29	30.16 ± 3.28
0.6	44.50 ± 1.50	04.28 ± 0.56	00.00	00.00	08.16 ± 0.53	02.28 ± 0.42	40.78 ± 2.01

Table 48: Differential Haemocyte Counts determined after 3 days following the topical application of various concentrations of Aminocarb on 1-2 day old 6th instar larvae of *Diacrisia obliqua*

Concentrations (%) 2 µl/larva	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Granulocytes % ± S. E.	Spherulocytes % ± S. E.	Oenocytoids % ± S. E.	Coagulocytes % ± S. E.	Disintegrating Cells % ± S. E.
Untreated	28.82 ± 1.51	53.10 ± 2.03	04.50 ± 0.89	02.18 ± 0.50	01.92 ± 0.65	02.08 ± 0.60	07.48 ± 1.21
Solvent Treated	25.54 ± 1.40	55.82 ± 2.42	05.54 ± 0.61	02.26 ± 0.52	00.88 ± 0.62	02.18 ± 0.70	07.78 ± 0.92
0.1	29.06 ± 2.05	50.70 ± 1.46	06.66 ± 0.50	01.40 ± 0.67	01.20 ± 0.68	03.54 ± 0.57	11.46 ± 1.25
0.2	37.68 ± 3.18	35.18 ± 2.79	02.28 ± 0.80	00.00	03.12 ± 0.74	03.96 ± 0.38	17.78 ± 2.85
0.4	48.14 ± 1.90	21.88 ± 3.86	00.00	00.00	03.90 ± 0.40	00.00	26.08 ± 3.13
0.6	32.64 ± 2.43	10.10 ± 1.07	00.00	00.00	07.68 ± 1.15	00.00	49.62 ± 2.41

Table 49: Differential Haemocyte Counts determined after 5 days following the topical application of various concentrations of Aminocarb on 1-2 day old 6th instar larvae of *Diacrisia obliqua*

Concentrations (%) 2 µl/larva	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Granulocytes % ± S. E.	Spherulocytes % ± S. E.	Oenocytoids % ± S. E.	Coagulocytes % ± S. E.	Disintegrating Cells % ± S. E.
Untreated	29016 ± 1.94	41.92 ± 2.79	12.74 ± 1.21	03.74 ± 0.79	02.10 ± 0.63	02.32 ± 0.75	08.02 ± 0.97
Solvent Treated	27.04 ± 1.55	43.50 ± 2.84	11.94 ± 1.17	03.70 ± 0.62	02.16 ± 0.48	02.46 ± 0.59	09.26 ± 1.23
0.1	31.16 ± 3.00	33.16 ± 3.81	13.66 ± 1.57	02.94 ± 0.74	03.06 ± 0.27	03.80 ± 0.99	12.26 ± 1.22
0.2	32.98 ± 1.80	26.50 ± 2.89	10.40 ± 1.28	02.04 ± 0.48	03.08 ± 0.36	03.40 ± 0.52	21.56 ± 1.51
0.4	35.42 ± 1.96	20.64 ± 1.67	04.24 ± 0.75	00.00	03.92 ± 0.66	03.96 ± 0.42	31.82 ± 0.90
0.6	38.76 ± 1.78	10.36 ± 0.88	00.00	00.00	07.08 ± 1.40	01.48 ± 0.72	42.38 ± 1.24

Table 50: Differential Haemocyte Counts determined in pupa following the topical application of various concentrations of Aminocarb on 1-2 day old 6th instar larvae of *Diacrisia obliqua*

Concentrations (%) 2 µl/larva	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Granulocytes % ± S. E.	Spherulocytes % ± S. E.	Oenocytoids % ± S. E.	Coagulocytes % ± S. E.	Disintegrating Cells % ± S. E.
Untreated	17.90 ± 2.53	72.90 ± 3.94	01.46 ± 0.72	00.00	00.00	01.10 ± 0.73	06.68 ± 0.94
Solvent Treated	14.48 ± 1.89	73.92 ± 1.67	02.42 ± 0.69	00.00	00.00	00.00	09.26 ± 0.87
0.1	20.48 ± 4.26	64.46 ± 3.03	03.50 ± 0.82	00.00	00.00	00.00	11.54 ± 1.89
0.2	22.86 ± 2.07	49.78 ± 3.32	03.40 ± 0.73	00.00	00.00	01.98 ± 0.66	20.24 ± 1.45
0.4	26.92 ± 5.08	42.04 ± 2.71	04.12 ± 0.36	00.00	00.00	00.00	26.78 ± 1.54
0.6	00.00	00.00	00.00	00.00	00.00	00.00	00.00

Table 51: Differential Haemocyte Counts determined after 6 hrs. following the topical application of various concentrations of cypermethrin on 1-2 day old 6th nstar larvae of *Diacrisia obliqua*

Concentrations (%) 2 µl/larva	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Granulocytes % ± S. E.	Spherulocytes % ± S. E.	Oenocytoids % ± S. E.	Coagulocytes % ± S. E.	Disintegrating Cells % ± S. E.
Untreated	31.34 ± 3.12	51.72 ± 3.47	03.26 ± 0.36	01.10 ± 0.52	01.84 ± 0.43	01.82 ± 0.56	08.94 ± 2.31
Solvent Treated	32.92 ± 1.24	49.32 ± 2.69	03.78 ± 0.55	01.38 ± 0.87	01.86 ± 0.43	01.08 ± 0.47	09.66 ± 0.92
0.0025	35.40 ± 2.45	46.24 ± 2.21	01.94 ± 0.42	01.00 ± 0.48	01.36 ± 0.38	02.02 ± 0.56	12.04 ± 1.42
0.005	37.12 ± 2.40	38.60 ± 3.20	03.36 ± 0.81	02.04 ± 0.45	02.14 ± 0.13	02.92 ± 0.59	13.82 ± 1.61
0.01	34.10 ± 1.67	18.58 ± 2.47	07.22 ± 0.91	00.00	04.24 ± 0.50	04.08 ± 0.38	31.82 ± 2.22
0.015	39.14 ± 2.33	13.24 ± 1.92	02.46 ± 0.68	00.00	08.16 ± 2.34	03.74 ± 0.45	33.26 ± 3.41

Table 52: Differential Haemocyte Counts determined after 1 day following the topical application of various concentrations of cypermethrin on 1-2 day old 6th instar larvae of *Diacrisia obliqua*

Concentrations (%) 2 µl/larva	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Granulocytes % ± S. E.	Spherulocytes % ± S. E.	Oenocytoids % ± S. E.	Coagulocytes % ± S. E.	Disintegrating Cells % ± S. E.
Untreated	37.14 ± 2.40	49.08 ± 2.88	06.14 ± 0.73	01.22 ± 0.47	01.04 ± 0.46	02.20 ± 0.50	03.18 ± 0.55
Solvent Treated	35.66 ± 2.39	51.52 ± 2.99	04.64 ± 0.54	02.14 ± 0.41	01.00 ± 0.48	01.04 ± 0.45	04.06 ± 0.94
0.0025	34.14 ± 3.76	42.84 ± 3.02	07.78 ± 1.45	01.22 ± 0.42	01.92 ± 0.56	00.86 ± 0.36	11.30 ± 0.41
0.005	38.70 ± 2.64	27.44 ± 0.69	06.10 ± 1.33	02.08 ± 0.58	03.04 ± 0.50	02.86 ± 0.58	16.04 ± 1.21
0.01	43.08 ± 3.55	17.32 ± 1.26	00.00	00.00	04.12 ± 0.69	02.26 ± 0.66	33.26 ± 2.40
0.015	45.44 ± 1.99	05.10 ± 0.91	00.00	00.00	04.36 ± 0.40	02.68 ± 0.63	42.46 ± 1.59

Table 53: Differential Haemocyte Counts determined after 3 days following the topical application of various concentrations of cypermethrin on 1-2 day old 6th instar larvae of *Diacrisia obliqua*

Concentrations (%) 2 µl/larva	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Granulocytes % ± S. E.	Spherulocytes % ± S. E.	Oenocytoids % ± S. E.	Coagulocytes % ± S. E.	Disintegrating Cells % ± S. E.
Untreated	30.02 ± 2.63	53.70 ± 1.84	05.36 ± 0.66	02.22 ± 0.37	02.04 ± 0.36	01.20 ± 0.55	05.42 ± 0.70
Solvent Treated	27.76 ± 0.97	56.90 ± 2.95	04.04 ± 0.61	01.54 ± 0.45	01.42 ± 0.45	01.46 ± 0.44	06.86 ± 1.10
0.0025	32.60 ± 2.33	44.50 ± 2.55	02.98 ± 0.53	02.04 ± 0.63	02.18 ± 0.61	01.94 ± 0.28	13.90 ± 1.29
0.005	35.52 ± 1.76	40.54 ± 2.05	00.00	00.00	02.06 ± 1.54	02.32 ± 0.67	19.62 ± 1.65
0.01	40.16 ± 1.77	26.88 ± 1.82	00.00	00.00	03.98 ± 0.59	01.96 ± 0.40	27.06 ± 2.80
0.015	28.06 ± 2.65	14.00 ± 1.00	00.00	00.00	04.02 ± 0.58	02.38 ± 0.38	27.04 ± 2.46

Table 54:Differential Haemocyte Counts determined after 5 days following the topical application of various concentrations of cypermethrin on 1-2 day old 6th instar larvae of *Diacrisia obliqua*

Concentrations (%) 2 µl/larva	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Granulocytes % ± S. E.	Spherulocytes % ± S. E.	Oenocytoids % ± S. E.	Coagulocytes % ± S. E.	Disintegrating Cells % ± S. E.
Untreated	33.98 ± 0.97	42.94 ± 1.46	09.16 ± 0.73	03.34 ± 0.36	01.64 ± 0.72	00.50 ± 0.33	08.42 ± 0.84
Solvent Treated	29.46 ± 1.84	47.12 ± 3.47	10.04 ± 1.52	02.82 ± 0.40	01.08 ± 0.47	01.38 ± 0.60	08.06 ± 1.32
0.0025	34.04 ± 1.84	40.62 ± 2.38	10.08 ± 1.24	01.36 ± 0.43	02.00 ± 0.34	00.96 ± 0.42	10.96 ± 0.86
0.005	35.52 ± 1.66	35.31 ± 2.45	04.50 ± 0.73	00.00	03.00 ± 0.53	00.00	21.66 ± 0.91
0.01	38.38 ± 1.68	27.42 ± 3.89	00.00	00.00	03.40 ± 0.77	01.80 ± 0.54	29.00 ± 1.66
0.015	39.60 ± 1.05	19.16 ± 3.20	00.00	00:00	04.38 ± 0.48	02.10 ± 0.57	34.82 ± 2.47

Table 55: Differential Haemocyte Counts determined in pupa following the topical application of various concentrations of cypermethrin on 1-2 day old 6th instar larvae of *Diacrisia obliqua*

Concentrations (%) 2 µl/larva	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Granulocytes % ± S. E.	Spherulocytes % ± S. E.	Oenocytoids % ± S. E.	Coagulocytes % ± S. E.	Disintegrating Cells % ± S. E.
Untreated	15.74 ± 1.33	73.87 ± 2.55	02.24 ± 0.59	Absent	Absent	00.58 ± 0.26	07.62 ± 1.54
Solvent Treated	14.28 ± 0.36	76.50 ± 1.12	01.92 ± 0.59	-do-	-do-	00.00	07.38 ± 0.73
0.0025	16.72 ± 1.86	68.60 ± 2.11	04.20 ± 0.79	-do-	-do-	00.00	10.52 ± 0.60
0.005	20.72 ± 1.51	57.58 ± 1.60	04.26 ± 0.67	-do-	-do-	01.12 ± 0.51	16.38 ± 3.15
0.01	00.00	00.00	00.00	-do-	-do-	00.00	00.00
0.015	00.00	00.00	00.00	-do-	-do-	00.00	00.00

Table 56: Differential Haemocyte Counts determined after 6 hrs. following the topical application of various concentrations of Muristerone on 1-2 day old 6th instar larvae of *Diacrisia obliqua*

Concentrations (%) 2 µl/larva	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Granulocytes % ± S. E.	Spherulocytes % ± S. E.	Oenocytoids % ± S. E.	Coagulocytes % ± S. E.	Disintegrating Cells % ± S. E.
Untreated	31.98 ± 1.43	54.68 ± 3.13	01.98 ± 0.66	01.90 ± 0.53	02.10 ± 0.60	02.96 ± 0.33	04.40 ± 0.92
Solvent Treated	35.00 ± 1.76	51.10 ± 2.73	03.18 ± 0.73	02.86 ± 0.24	00.94 ± 0.42	01.78 ± 0.22	05.16 ± 0.58
0.5	30.72 ± 3.26	48.40 ± 2.47	03.86 ± 0.75	02.90 ± 0.43	02.28 ± 0.61	02.40 ± 0.39	09.48 ± 0.76
1.0	25.82 ± 1.56	48.98 ± 2.36	05.10 ± 0.92	03.42 ± 0.57	02.24 ± 0.64	03.06 ± 0.57	11.38 ± 0.72
1.5	24.96 ± 1.45	42.16 ± 2.00	05.84 ± 0.66	02.98 ± 0.44	02.18 ± 0.35	02.02 ± 0.18	19.88 ± 1.76
2.0	21.42 ± 2.44	42.06 ± 2.83	07.46 ± 1.07	03.86 0.46	03.02 ± 0.12	00.00	22.22 ± 2.18

Table 57: Differential Haemocyte Counts determined after 1 day following the topical application of various concentrations of Muristerone on 1-2 day old 6th instar larvae of *Diacrisia obliqua*

Concentrations (%) 2 µl/larva	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Granulocytes % ± S. E.	Spherulocytes % ± S. E.	Oenocytoids % ± S. E.	Coagulocytes % ± S. E.	Disintegrating Cells % ± S. E.
Untreated	29.14 ± 1.72	51.96 ± 2.51	05.28 ± 0.60	02.26 ± 0.43	01.54 ± 0.49	00.98 ± 0.41	05.94 ± 0.85
Solvent Treated	31.04 ± 3.54	49.90 ± 4.28	04.84 ± 0.53	02.26 ± 0.83	02.18 ± 0.41	01.60 ± 0.46	08.24 ± 0.89
0.5	34.20 ± 1.50	43.38 ± 1.54	07.16 ± 0.51	01.36 ± 0.42	02.02 ± 0.44	02.22 ± 0.32	09.66 ± 0.90
1.0	36.40 ± 2.13	35.62 ± 2.35	11.40 ± 1.50	01.36 ± 0.59	02.24 ± 0.83	02.72 ± 0.51	10.30 ± 0.66
1.5	36.26 ± 0.89	28.98 ± 1.82	13.40 ± 1.55	00.00	02.60 ± 0.25	02.98 ± 0.79	15.82 ± 1.16
2.0	37.72 ± 2.35	24.02 ± 3.04	13.24 ± 0.73	00.00	02.96 ± 0.57	04.06 ± 0.65	17.44 ± 3.12

Table 58: Differential Haemocyte Counts determined after 3 day following the topical application of various concentrations of Muristeron on 1-2 day old 6th instar larvae of *Diacrisia obliqua*

Concentrations (%) 2 µl/larva	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Granulocytes % ± S. E.	Spherulocytes % ± S. E.	Oenocytoids % ± S. E.	Coagulocytes % ± S. E.	Disintegrating Cells % ± S. E.
Untreated	22.44 ± 1.39	53.52 ± 3.21	08.62 ± 1.07	03.64 ± 1.28	02.10 ± 0.64	03.34 ± 0.62	06.38 ± 0.59
Solvent Treated	24.64 ± 1.03	52.14 ± 1.54	09.06 ± 1.57	03.90 ± 0.72	01.30 ± 0.39	02.74 ± 0.43	06.26 ± 0.44
0.5	27.08 ± 2.05	49.56 ± 2.13	08.10 ± 1.08	02.88 ± 0.30	02.24 ± 0.42	03.06 ± 0.58	07.16 ± 0.67
1.0	28.80 ± 1.68	42.38 ± 1.71	12.02 ± 1.04	01.86 ± 0.55	02.54 ± 0.43	03.78 ± 0.53	08.66 ± 1.17
1.5	32.12 ± 1.75	31.46 ± 1.36	12.24 ± 1.03	00.00	03.84 ± 0.55	04.42 ± 0.61	15.92 ± 1.57
2.0	32.92 ± 1.90	26.06 ± 1.76	10.74 ± 0.53	00.00	04.02 ± 0.54	04.70 ± 0.23	21.60 ± 2.18

Table 59: Differential Haemocyte Counts determined after 5 days following the topical application of various concentrations of Muristerone on 1-2 day old 6th instar larvae of *Diacrisia obliqua*

Concentrations (%) 2 µl/larva	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Granulocytes % ± S. E.	Spheruloeytes % ± S. E.	Oenocytoids % ± S. E.	Coagulocytes % ± S. E.	Disintegrating Cells % ± S. E.
Untreated	18.26 ± 2.10	56.10 ± 1.55	12.88 ± 0.98	02.96 ± 1.00	01.76 ± 0.33	04.12 ± 0.50	03.94 ± 0.16
Solvent Treated	22.00 ± 0.86	51.80 ± 2.33	11.82 ± 0.80	02.92 ± 0.35	02.06 ± 0.58	03.76 ± 0.68	05.66 ± 1.28
0.5	21.90 ± 2.32	49.90 ± 0.95	10.74 ± 0.71	02.04 ± 0.58	02.64 ± 0.81	03.80 ± 0.67	09.00 ± 0.97
1.0	26.32 ± 1.28	43.80 ± 2.30	07.80 ± 0.92	02.28 ± 0.51	02.92 ± 0.43	04.88 ± 0.49	12.04 ± 0.69
1.5	27.00 ± 1.50	38.62 ± 1.53	07.72 ± 0.90	00.00	04.20 ± 0.44	06.04 ± 0.38	16.56 ± 0.74
2.0	00.00	00.00	00.00	00.00	00.00	00.00	00.00

Table 60: Differential Haemocyte Counts determined in pupa following the topical application of various concentrations of Muristerone on 1-2 day old 6th instar larvae of *Diacrisia obliqua*

Concentrations (%) 2 µl/larva	Prohaemocytes % ± S. E.	Plasmatocytes % ± S .E.	Granulocytes % ± S. E.	Spherulocytes % ± S. E.	Oenocytoids % ± S. E.	Coagulocytes % ± S. E.	Disintegrating Cells % ± S. E.
Untreated	18.86 ± 2.15	69.48 ± 1.03	02.90 ± 0.54	Absent	Absent	01.86 ± 0.58	06.92 ± 2.02
Solvent Treated	17.74 ± 1.97	72.44 ± 1.88	02.08 ± 0.39	-do-	-do-	02.06 ± 0.43	05.68 ± 0.56
0.5	21.88 ± 2.29	61.28 ± 6.84	04.40 ± 0.70	-do-	-do-	02.82 ± 0.48	09.68 ± 1.21
1.0	24.18 ± 1.78	57.48 ± 1.94	04.82 ± 1.58	-do-	-do-	03.62 ± 0.68	09.88 ± 1.44
1.5	00.00	00.00	00.00	-do-	-do-	00.00	00.00
2.0	00.00	00.00	00.00	-do-	-do-	00.00	00.00

Table 61: Differential Haemocyte Counts determined after 6 hrs. following the topical application of various concentrations of Methoprene on 1-2 day old 6th instar larvae of *Diacrisia obliqua*

Concentrations (%) 2 µl/larva	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Granulocytes % ± S. E.	Spherulocytes % ± S. E.	Oenocytoids % ± S. E.	Coagulocytes % ± S. E.	Disintegrating Cells % ± S. E.
Untreated	31.04 ± 1.22	55.34 ± 2.06	02.02 ± 0.41	01.70 ± 0.59	01.14 ± 0.36	03.12 ± 0.36	05.58 ± 0.81
Solvent Treated	33.72 ± 1.24	51.24 ± 1.36	03.12 ± 0.44	02.08 ± 0.36	01.92 ± 0.25	02.88 ± 1.46	05.04 ± 0.44
0.4	30.98 ± 2.16	47.60 ± 2.90	03.92 ± 0.29	03.16 ± 0.56	02.94 ± 0.36	03.08 ± 0.36	08.34 ± 0.64
0.6	30.40 ± 1.19	47.36 ± 2.15	04.78 ± 0.92	03.04 ± 0.43	01.96 ± 0.53	03.04 ± 0.69	09.42 ± 0.85
0.8	26.66 ± 1.45	43.88 ± 3.10	05.68 ± 0.57	02.14 ± 0.57	02.86 ± 0.52	03.76 ± 0.45	15.06 ± 0.98
1.0	23.10 ± 2.19	44.62 ± 1.77	07.82 ± 0.78	02.36 0.68	03.64 ± 0.41	01.84 ± 0.59	16.72 ± 1.74

Table 62: Differential Haemocyte Counts determined after 1 day following the topical application of various concentrations of Methoprene on 1-2 day old 6th instar larvae of *Diacrisia obliqua*

Concentrations (%) 2 µl/larva	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Granulocytes % ± S. E.	Spherulocytes % ± S. E.	Oenocytoids % ± S. E.	Coagulocytes % ± S. E.	Disintegrating Cells % ± S. E.
Untreated	30.52 ± 2.10	52.42 ± 2.26	04.34 ± 0.49	02.30 ± 0.47	01.06 ± 0.21	01.96 ± 0.21	07.62 ± 0.43
Solvent Treated	33.82 ± 1.58	49.36 ± 1.84	03.72 ± 0.36	02.24 ± 0.32	01.30 ± 0.38	02.16 ± 0.21	07.46 ± 0.47
0.4	38.86 ± 3.41	42.16 ± 1.78	07.10 ± 0.97	00.00	01.72 ± 0.16	02.04 ± 0.25	08.16 ± 0.52
0.6	41.30 ± 2.08	36.86 ± 1.37	04.24 ± 0.90	00.00	02.10 ± 0.57	02.94 ± 0.35	12.58 ± 1.35
0.8	41.96 ± 3.76	33.40 ± 3.09	02.98 ± 0.50	00.00	02.90 ± 0.53	02.96 ± 0.61	15.88 ± 1.25
1.0	42.46 ± 2.85	26.74 ± 1.29	02.86 ± 0.46	00.00	03.16 ± 0.23	03.88 ± 0.10	20.30 ± 2.00

Table 63: Differential Haemocyte Counts determined after 3 days following the topical application of various concentrations of Methoprene on 1-2 day old 6th instar larvae of *Diacrisia obliqua*

Concentrations (%) 2 µl/larva	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Granulocytes % ± S. E.	Spherulocytes % ± S. E.	Oenocytoids % ± S. E.	Coagulocytes % ± S. E.	Disintegrating Cells % ± S. E.
Untreated	23.12 ± 0.84	51.24 ± 2.25	09.26 ± 0.50	03.80 ± 0.65	01.92 ± 0.28	04.06 ± 0.89	06.64 ± 1.02
Solvent Treated	22.66 ± 2.42	54.34 ± 3.01	09.06 ± 1.18	02.90 ± 0 52	01.84 ± 0.21	03.00 ± 0.55	06.26 ± 0.99
0.4	24.92 ± 2.35	46.48 ± 2.36	12.16 ± 0.88	02.06 ± 0.46	02.30 ± 0.57	03.02 ± 0.51	08.92 ± 0.81
0.6	26.90 ± 1.78	43.32 ± 2.31	12.64 ± 1.45	02.04 ± 0.42	02.10 ± 0.29	02.06 ± 0.35	10.94 ± 0.75
0.8	29.08 ± 2.29	35.00 ± 2.43	14.64 ± 1.07	01.22 ± 0.33	02.58 ± 0.18	01.38 ± 0.19	16.12 ± 0.57
1.0	30.22 ± 2.00	31.56 ± 1.14	13.58 ± 0.67	00.00	03.12 ± 0.45	00.00	21.54 ± 1.51

Table 64: Differential Haemocyte Counts determined after 5 days following the topical application of various concentrations of Methoprene on 1-2 day old 6th instar larvae of *Diacrisia obliqua*

Concentrations (%) 2 µl/larva	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Granulocytes % ± S. E.	Spherulocytes % ± S. E.	Oenocytoids % ± S. E.	Coagulocytes % ± S. E.	Disintegrating Cells % ± S. E.
Untreated	18.32 ± 2.02	54.84 ± 2.57	13.10 ± 1.12	03.10 ± 0.56	01.92 ± 0.37	04.18 ± 0.29	04.60 ± 0.61
Solvent Treated	18.82 ± 1.04	53.86 ± 1.22	11.22 ± 0.56	02.86 ± 0.10	02.28 ± 0.35	03.64 ± 0.55	06.48 ± 1.04
0.4	20.60 ± 1.78	50.58 ± 2.73	10.16 ± 0.48	03.18 ± 0.57	02.16 ± 0.42	03.82 ± 0.30	09.16 ± 1.12
0.6	25.26 ± 1.67	46.34 ± 2.77	08.04 ± 1.07	02.08 ± 0.47	03.38 ± 0.62	02.98 ± 0.49	11.94 ± 0.53
0.8	40.28 ± 2.48	30.42 ± 2.60	06.94 ± 0.86	02.08 ± 2.24	03.70 ± 0.24	02.14 ± 0.53	14.34 ± 0.63
1.0	43.04 ± 2.32	30.64 ± 3.21	03.70 ± 0.48	00.00	04.20 ± 0.67	01.26 ± 0.43	17.20 ± 0.29

Table 65: Differential Haemocyte Counts determined in pupa following the topical application of various concentrations of Methoprene on 1-2 day old 6th instar larvae of *Diacrisia obliqua*

Concentrations (%) 2 µl/larva	Prohaemocytes % ± S. E.	Plasmatocytes % ± S. E.	Granulocytes % ± S. E.	Spherulocytes % ± S. E.	Oenocytoids % ± S. E.	Coagulocytes % ± S. E.	Disintegrating Cells % ± S. E.
Untreated	13.60 ± 0.65	70.64 ± 1.05	09.00 ± 0.86	00.00	00.00	02.22 ± 0.17	04.54 ± 0.42
Solvent Treated	11.78 ± 0.59	69.74 ± 1.65	11.86 ± 0.83	00.00	00.00	02.68 ± 0.48	03.94 ± 0.36
0.4	08.02 ± 0.46	71.84 ± 1.24	07.04 ± 0.33	01.38 ± 0.43	00.00	02.14 ± 0.38	09.02 ± 0.89
0.6	05.86 ± 0.66	74.90 ± 1.51	01.88 ± 0.52	01.28 ± 0.38	00.00	03.82 ± 0.26	13.18 ± 0.57
0.8	00.00	00.00	00.00	00.00	00.00	00.00	00.00
1.0	00.00	00.00	00.00	00.00	00.00	00.00	00.00

V- DISCUSSION

Free haemocytes under normal conditions

Among arthropods haemocytes have been most extensively studied in insects. A uniform, widely acceptable haemocyte classification is still lacking for insects as well as for other arthropods. Substantial disagreement exists regarding not only the classification of haemocytes but their numbers as well. The disagreement among different authors is, in part, due to the use of different parameters e.g. method of cell preparation, stage of insect development, physiological state of individual insect, type of microscopical method used, inconsistencies in nomenclature and variation in the experimental technique adopted. Consequently, confusion exists regarding the adopted terminology as well as use of synonyms for the same cell type (Yeager 1945, Jones 1962, Gupta 1979). A systematic characterization by adopting two or more complementary approaches is more than overdue.

The insect haemocyte classification that is generally used has evolved over more than half a century. According to Millara (1947), Cue'not, in 1896, was the first to classify insect haemocytes into four categories and was later followed in this attempt by Hollande (1909, 1911) and others. Wigglesworth (1939) summarized most of the earlier classifications and modified it later (1959). Yeager (1945) gave a more elaborate classification of haemocytes. Jones (1962) revised and greatly improved Yeager's classification. Based on their morphology and cellular structure 9 types of haemocytes were eventually recognized (Jones 1962, 1965). Price and Ratcliffe (1974) proposed 6 main types based on twenty-eight species representing 15 insect orders. Several hemipteran and lepidopteran species possess 5 to 6 cell types.

In the present investigation, the free haemocytes of *Dysdercus cingulatus* and *Diacrisia obliqua* were identified and classified on the basis of variations in their staining reactions, cell inclusions, shape and size. Therefore, 5 types of haemocytes viz., prohaemocyte, plasmatocyte, granulocyte, adipohaemocyte and oenocytoid have been identified in the last instar nymphs and adults of *Dysdercus cingulatus* under light

microscope. Whereas, in the last larval instar of *Diacrisia obliqua*, 6 types of haemocytes have been described. These were prohaemocyte, plasmatocyte, granulocyte, spherulocyte, coagulocyte and oenocytoid. However, haemolymph of pupae of *D. obliqua* consisted of generally prohaemocytes, plasmatocytes and some times granulocytes and coagulocytes. The difference in the free haemocyte types of *Dysdercus cingulatus* and *Diacrisia obliqua* may be due to their growth, developmental state and food habits. The prohaemocytes, plasmatocytes, granulocytes and oenocytoids are the common variety of haemocytes among *D. cingulatus* and *D. obliqua*. Majority of species of insects studied so far also contain these cells in their haemolymph.

The cytoplasm of the prohaemocytes in the present species is usually scanty and basophilic. These cells are larger in *D. cingulatus* than in *Diacrisia obliqua*. Like in other insects, these cells are smaller in size as compared to other haemocytes, with nucleus almost occupying the whole cell. The term prohaemocytes was introduced by Arnold (1952). Prohaemocytes are regarded as stem cells which give rise to other cells. Functionally, these are transitory cells that are transformed into other types of haemocytes on demand (Crossley 1975). The term prohaemocytes used in the present study is compatible and similar to the previous reports in *Ctenolepisma longicaudata* (Pal and Chowdhury 1989); *Locusta migratoria* (Akai and Sato 1979); *Chrotogonus trachypterus* and *Acrida exaltata* (Sharma and Dutta 1979); *Schizodactylus monstrosus* (Islam and Roy 1982); *Hieroglyphus nigrorepletus* (Khan *et al* 1984, Ahmad 1986, Ahmad and Khan 1988); *Periplaneta americana* (Baerwald and Bousch 1970); *Leucophaea maderae* (Hagopian 1971); *Nauphoeta cinerea* (Kochetora 1978); *Panesthia angustipennis* (Akai and Sato 1979); *Dysdercus cingulatus* (Zaidi and Khan 1974); *Rhopalosiphum maidis* (Behura and Dash 1978); *Ephestia kuhniella* (Arnold 1952a); *Prodenia eridania* (Jones 1959); *Bombyx mori* (Nittono 1960); *Galleria mellonella* (Jones 1967); *Spodoptera litura* (Nishi 1982 and Ahmad 1986); *Spilosoma obliqua* (Ahmad 1986); *Heliothes armigera* (Khan *et al* 1990); *Argina astrea*, *Danais chrysippus*, and *Earias* (Saxena 1992); *Mythimna unipuncta* (Ribeiro *et al.* 1996); *Drosophila euronotus* (Nappi 1970); *Glossina austeri*, *G. morsitans*, *Aedes aegypti*, *Culex quinquefasciatus*, *Stomoxys calcitrans*, *Calliphora erythrocephala* and *Lucilia sericata* (Kaaya and Ratcliffe 1982); *Melolontha*

melolontha, *Mylabris pustulata* (Ahmad 1974); *Dermestis vulpinus*, *D. maculatus*, *Hybosorus illegari* and *Dineutes aerius* (Al Khalifa and Siddiqui 1985); and *Polistis hebroeus* (Ahmad 1986).

The above mentioned species universally contain another type of haemocyte known as plasmatocytes. The polymorphic plasmatocytes characteristically have more cytoplasm and comparatively smaller nuclei than the prohaemocytes. The plasmatocytes of *Dysdercus cingulatus* and *Diacrisia obliqua* have the characteristics of typical plasmatocytes, however, those of former species are slightly larger in size. Since Yeager and Munson, 1941, introduced the term plasmatocyte, these cells or their variant forms have also been described under such names as amoebocytes, giant cells, hyaline cells, lamellocytes, leucocytes, lymphocytes, micronucleocytes, nematocytes, phagocytes, podocytes, radiate cells and vermicytes etc. However, the terms amoebocytes and phagocytes used for plasmatocytes by Wigglesworth in *R. prolixus* are only conditionally applicable to limited forms of plasmatocytes which are either irregular or round but do form pseudopodia (Jones 1977). However, plasmatocytes have also been functionally referred to as immunocytes in the cockroach *Gromphadorhina portentosa* (Gupta 1985 a). Beside phagocytosis, some other functions also are attributed to plasmatocytes. In *Cecropia* pupae the plasmatocytes are involved in the formation of cellular membranes during wound healing (Clark and Harvey 1965). In *Rhodnius prolixus* plasmatocytes take part in the formation of basement membrane (Wigglesworth 1973). In Bombyx mori plasmatocytes have no phagocytic function, but participate in encapsulation and wound healing (Akai and Sato 1979).

The granulocytes of *D. cingulatus* and *D. obliqua* have mild basophilic cytoplasm containing numerous prominent eosinophilic granules which is the basis to differentiate these cells from plasmatocytes and to name them granulocytes. However, the granulocytes of *D. cingulatus* generally contain less granules in cytoplasm compared to *D. obliqua*. Cue'not (1896) described amoebocytes as characteristically containing granules in their cytoplasm. Since then, haemocytes containing granules have been described by several other names, such as adipohaemocytes, cystocytes (coagulocytes), hyaline cells, phagocytes, pycnoleucocytes and spherulocytes. Indeed

granulocytes have been widely misidentified and confused with spherulocytes, adipohaemocytes and coagulocytes (Gupta 1986). Such cells (granulocytes) have been referred to as immunocytes in the cockroach, *Gromphadorhina portentosa* (Gupta 1985a) after studying their role in defense. The phagocytic functions of these cells were reported by Crossley (1964) in *Calliphora erythrocephala*; Arnold (1970) in *Diploptera punctata*; Akai and Sato (1973) in *Agallia constricta*; Saxena (1992) in *Argina astrea*, *Danais chrysippus* and *Earias*. Furthermore, Brehlin *et al.* (1975) in *Locusta migratoria* observed their participation in encapsulation of foreign bodies and in wound healing. Hagopian (1971) suggested their involvement in melanization of encapsulated material in cockroach. The granulocytes of present species are in conformity to those cells in most of the species referred earlier which contain granules in their cytoplasm.

Oenocytoids of *Dysdercus cingulatus* and *Diacrisia obliqua* are easily distinguishable on the basis of their homogeneous basophilic cytoplasm and small eccentric nucleus. Oenocytoids are the largest blood cells in *D. obliqua*. These cells are usually round or oval in shape in the present species. Such cells were also described by Wigglesworth (1956); Lai-Fook (1970) and Le Blanc (1971) in *Rhodnius prolixus*; Zaidi and Khan (1975) in *Dysdercus cingulatus*; Boitean and Perron (1976) in *Macrosiphum euphorbidae*; Siddiqui (1978) in *Nepa cineria* and *Leptocorisa varicornis*; Mall and Gupta (1978) in *Nezara viridula*; Saxena (1992) in *Argina astrea*, *Danais chrysippus* and *Earias*; Devauchelle (1971) in *Melolontha melolontha*; Ahmad (1974) in *Aulacophora fovicolis*, *Mylabris pustulata* and Al Khalifa and Siddiqui (1985) in *Dermestis vulpinus*, *Dermestis maculatus*, *Hybosorus illegari* and *Dineutes aerius*. However, Behura and Dash (1978) in *Rhopalosiphum maidis* and Ahmad (1986) in *Polystis hebroeus* could not report these cells. Ahmad (1974) reported that in *A. fovicolis* and *M. Pustulata* these cells were always found scattered and never in clusters as observed by Wigglesworth (1956) in *R. prolixus*. She also observed binucleate forms of oenocytoids in *Spilosoma obliqua (D. obliqua)*, *Spodoptera litura* and *Hieroglyphus nigrorepletus*. The origin and function of these cells is not yet known and the name oenocytoid has no relationship with oenocytes which are ectodermal in origin. However, oenocytoids are characteristically also comparable to the crescent cells in *Gromphadorhina portentosa*. Ritter (1965), who erroneously considered them

as unusual anucleate cells. These cells were later identified as oenocytoids by Gupta (1985a).

Adipohaemocytes were identified in nymphs and adults of *Dysdercus cingulatus* and were predominant type in adult males. Whereas, these haemocytes were absent in *Diacrisia obliqua*. These are the largest of all haemocytes of *Dysdercus cingulatus*, containing fat globules in the cytoplasm and relatively small nucleus which was central or eccentric in position. These fat containing cells have been observed in many insect species. Wigglesworth (1955) in *Rhodnius prolixus* termed these cells as adipocytes or lipocytes but Jones (1965) preferred to call these cells as adipohaemocyte in the same species. The argument of Jones for naming these cells is based on their homology with the term haemocytes. The adipohaemocytes were also reported by Islam and Roy (1982) in *Shizodactylus monstrosus*; Nishi (1982) and Khan *et al* (1984) in *Spodoptera litura;* Ahmad (1986) in 3rd and 4th instar larvae and pupae of *S. litura* and in pupae and adults of *Spilosoma obliqua*; Khan *et al.*, (1990) in *Heliothis armigera*; Kayya and Ratcliffe (1982) in *Glossina austeni*, *G. morsitans*, *Aedes aegypti*, *Culex quinquefasciatus*, *Stomoxys. calcitrans*, *Calliphora erythrocephala* and *Lucilia sericata*; Al-Khalifa and Siddiqui (1985) in *Dermestes vulpinus*, *D. naculatus*, *Hybosorus illegeri* and *Dineutes aerius*; Siddiqui (1990) in *Leptocorisa varicornis*, *Nepa cinerea* and *Ademsia cancellata*. But adipohaemocytes could not be observed by Akai and Sato (1979) in *Locusta migratoria*; Goffinet and Gregoire (1979) in *Gryllotalpa* sp.; Behura and Dash (1978) in *R. maidis*; Nittono (1960) in adult *Bombyx mori*; Jones (1967) in *Galleria mellonella*; Zachary and Hoffman (1973) and Jones (1950) in *Tenebrio molitor*; Ribeiro (1996) in *Mythimna unipuncta*.

The spherulocytes are characteristically evident by their conspicuous spherules, filling the cytoplasm. In the present investigation these cells were identified only in *Diacrisia obliqua* larvae and were absent in *Dysdercus cingulatus*. Similar cells have been reported in *Calliphora erythrocephala* (Akesson 1953); *Sarcophaga bullata* (Jones 1956); Cockroaches (Wigglesworth 1959; Gupta and Sutherland 1967; Arnold 1970; Moran 1971; Scharrer, 1972); *Bombyx mori* (Nittono 1960 and Akai and Sato 1973); *Panesthia angustipennis* and *Holotrichia kiotoensis* (Akai and Sato 1979).

Aulacophora foevicolis and *Spodoptera litura* (Ahmad 1986), *Argina astrea*, *Danais chrysippus* and *Earias* (Saxena 1992). Adequate information about the function of spherulocytes is lacking. Wigglesworth (1959) suggested that they carry hormones. Nittono (1960) suggested their involvement in silk production in different strains of silk worm. Ashhurst (1982) proposed that these cells prevent coagulation of the haemolymph.

The coagulocytes were observed in larvae and pupae of *Diacrisia obliqua*. These cells were identified on the basis of having hyaline cytoplasm. These cells were not observed in *D. cingulatus*. Jones (1959) suggested that these cells were comparable to granulocytes of other insects. In a number of insect species such cells were called "Large hyaline haemocytes" (Gregoire 1951). Similar cells were observed in *Locusta migratoria* (Hoffman *et al.*, 1969; Akai and Sato 1979), *Argina astrea, Danais chrysippus* and *Earias* (Saxena 1992). In *Melolontha melolontha* and *L. migratoria*, Brehlin *et al.*, (1975) also reported that coagulocytes show phagocytosis. Gregoire (1970) reported their involvement in coagulation of insect blood.

Normal haemocytes under TEM

The present study of the ultrastructure of free haemocytes of fully grown larvae of *Diacrisia obliqua* revealed constant occurrence of only five cell types as against six types under light microscope, each having characteristic features with regard to morphology and cell organelles. However, intermediate forms also were frequently found which probably indicate relationship between certain cell types. The identification and characterization of haemocytes of the present species at the ultrastructural level support the criteria of Akai and Sato (1971), Lai-Fook (1973), Neuwirth (1973), Gupta (1979), Rowley and Ratcliffe (1981). Crossley (1964) suggested that classification based solely on morphology could be misleading. This view was shared by Scharrer (1966). Later, some researchers favoured the concept of ultrastructural integrity of various haemocyte types (Akai and Sato 1973, Lai-Fook 1973, Neuwirth 1973 and Raina 1976) as the rational basis of the haemocyte classification in insects.

In the present species, *D. obliqua,* the larval prohaemocytes represented similar features as described in other species. Their ultrastructure was further characterized by the presence of relatively small number of cytoplasmic organelles, absence of granules and pinocytotic vacuoles suggesting their non-involvement in phagocytic activity. Therefore, the prohaemocytes of present species are not the subject of any controversy with other species of insects.

The plasmatocytes are also known to occur in almost all insects studied so far, though sometimes differently named but generally having the same histological appearance. Since, these cells occur in different shapes, they are commonly regarded as polymorphs. It is one of the more common cell types. In *D. obliqua* also the plasmatocytes represented the well known characteristics. Plasmatocytes together with granulocytes, are the haemocyte types, that has led to the greatest dispute. In Lepidoptera they usually do not exhibit peculiar inclusions which was also the case in the present study of the haemocytes of *D. obliqua*. On the other hand, in other species, haemocytes with homogeneous dense granules have also been called "Plasmatocytes" (Ratcliffe and Rowley 1981).

Brehlin *et al.,* (1978) and Brehlin and Zachary (1983) distinguished two plasmatocyte types viz macrophages and typical plasmatocytes. The former subtype is characterized with numerous primary and secondary lysosomes and resorptive bodies which exhibit substantial endocytotic capacities *in vivo*, whereas, the typical plasmatocytes do not possess numerous lysosomes, resorptive bodies or pinocytotic vesicles. But Essawy *et al.*, (1985) found only second subtype of plasmatocytes in *Heliothis armigera*. In the present study on *D. obliqua*, one or two lysosomes were almost always present in the cytoplasm of the plasmatocytes, whereas, the pinocytotic vesicles were present near the periphery of the cytoplasm just beneath the cell membrane. Therefore, it is not possible to group these cells in sub-types in this species. However, the presence of lysosomes, resorptive bodies and pinocytotic vesicles in the plasmatocytes of *Diacrisia obliqua* theoretically gives a hint of their phagocytic functions, on the basis of the demonstration of phagocytic activity by the plasmatocytes with such inclusions in *Galleria mellonella* (Ratcliffe and Rowley 1974, Rowley and Ratcliffe 1976 b) and *Calliphora erythrocephala* (Rowley and Ratcliffe

1976 a). On the other hand, in *Calpodes ethlius* the granulocytes were the main phagocytic cells whereas the plasmatocytes appeared to be non-phagocytic in function (Neuwirth 1973). The difference in the haemocyte types with regard to phagocytic function has been attributed to varying factors by Jones (1962), Neuwirth (1973) and Price and Ratcliffe (1974). In *Mythimna unipunta* the plasmatocytes were found to build capsules around foreign body (Ribeiro 1996).

The greatest confusion regarding haemocyte terminology refers to the haemocytes having cytoplasmic granules. The granulocytes, as presently described in *D. obliqua* were earlier also labeled as adipohaemocytes in *Galleria* (Ashhurst and Richards 1964 and Jones 1967), plasmatocytes in *R. prolixus* (Lai-Fook 1970) and stem cells, clotting cells and phagocytic amoebocytes in *Tenebrio* (StangVoss 1970). As referred by Neuwirth (1973) these may be regarded the same cell type and variation may be attributed to fixation. The present haemocyte pattern of *D. obliqua* differentiates granulocytes from plasmatocytes on account of the presence of granules which may be densely or loosely distributed in the former cells. Presence of small granules near Golgi complex and endoplasmic reticulum gives an indication of involvement of the organelles in the formation of the granules. A variation in the density of these granules could be interpreted as an indication of their different maturation stages. On the basis of presence of differently sized granules and their electron density, Joshi and Lambdin (1996) classified granulocytes into T-granulocytes and M-granulocyte representing typical and modified forms respectively. They further pointed out that the number of T-granulocytes as well as number of granules within them were comparatively smaller in *Dactylopius* than described in *Calpodes* sp (Lai-Fook 1973) or in *Galleria* sp. (Neuwirth 1973). Similarly, in the present species (*Diacrisia obliqua*) number of granules in granulocytes was rather low and the size was generally smaller, though, in some cells large granules were also present but they were very few. According to Lai-Fook (1973) the granules appear to be formed and enlarged through the fusion of vesicles derived from the Golgi complex. In the present study also, Golgi complex and endoplasmic reticulum have been observed to be involved in the formation of these granules. The close association of these organelles and granules as observed in the present case, is in conformity with previous records (Lai Fook 1973, Neuwirth 1973, Bodammer 1978, Joshi and Lambdin 1996, Ribeiro

1996). In addition to these granules, various clusters of numerous fine secretory granules were also observed in the cytoplasm of granulocytes of *D. obliqua*. Morphologically similar secretory granules were also the main distinctive feature of M-granulocytes of *Dactylopius* (Joshi and Lambdin 1996). The above-mentioned authors on the basis of positive biochemical test with PAS reagent inferred that these secretory granules are either glycoproteins or glycogen complexes. Highly organized and well developed sub-marginal network of rough endoplasmic reticulum, peripheral accumulation of fine secretory granules and secretion/deposition of the secretory granule complexes into the haemolymph prompted Joshi and Lambdin (1996) to suggest that M-granulocytes adopted special function of synthesis and secretion of these fine secretory granules in *D. confusus*. One of the undisputed functions of the granulocytes is phagocytosis which has been experimentally demonstrated by Akai and Sato (1973) in *Bombyx mori* and by Neuwirth (1974) in *Calpodes*. The cytoplasm of granulocytes generally contains lysosomes which are indicative of their phagocytic function. In the present species also, the granulocytes were found to contain many lysosomes. According to Crossley (1964), the lysosomes produce enzymes for the breakdown of phagocytized material. Taylor (1969) suggested that the granules are analogous in structure and function to vertebrate premelanosomes. However, Lai-Fook (1973) and Neuwirth (1974), in *Calpodes* and *Galleria* respectively, demonstrated that granules were not premelanosomes because of a negative phenoloxidase reaction. Baerwald and Bousch (1970) merely referred to the granules as unstructured inclusions.

In *D. obliqua* the spherulocytes were infrequent and sometimes difficult to distinguish from granulocytes. In cockroaches, spherule cells or spherulocytes, a distinctive form of granular haemocyte, occur in most modern species, but were absent from most species in the more primitive group (Arnold 1972). The electron micrograph of the mature larvae of *B. mori* showed that the granular materials from mature spherule cells are sometimes released into the haemolymph (Akai and Sato 1973). However, some strains of *Bombyx* larvae lack spherule cells and have the tendency to produce less silk (Nittono 1960). In the present insect, *D. obliqua*, the spherulocytes contained smaller and fewer spherules than the spherulocytes described in other lepidopteran species i.e. *Spodoptera littoralis* (Harpaz *et al.*, 1969),

Bombyx mori (Akai and Sato 1973), *Calpodes ethlius* (Lai-Fook 1973), *Galleria mellonella* (Neuwirth 1973, Ashhurst 1982), *Antheraea perni* (Beaulaton & Monpeyssin 1976), *Cirplus unipunctata* (Brehlin 1977) and *Heliothis armigera* (Essawy *et. al.* 1985) etc. However, despite the fact that the spherules can exhibit different ultrastructural features, most of the authors are in agreement concerning the description and naming of these cell types. But Harpaz *et al.*, (1969) called them adipohaemocytes containing the spherules. However, no evidence is presented for the lipid nature of the spherules in these cells. The function of spherules has not been clearly understood yet. These cells may be involved in storing the acidic mucosubstances which were released into haemolymph according to the requirement.

Oenocytoids have been recorded in most of the insect species studied to date. As in the larvae of *D. obliqua*, these haemocytes are quite distinct from other blood cells and universally represent the characteristics of large size, eccentric nucleus, homogenous fibrous cytoplasm and paucity of cytoplasmic organelles. In some species such as *Bombyx mori* (Akai and Sato 1973) and *Galleria mellonella* (Lai-Fook 1970), these cells have a tubular structure in the hyaloplasm. In *Heliothis armigera* and *Cirplus unipunctata*, hyaloplasm of oenocytoids presented regular areas with less opacity to electrons (Essawy *et al.*, 1985, and Brehlin 1977 respectively). In *D. obliqua*, the oenocytoids have cytoplasm of uniform consistency with occasional organelles near the periphery of the cell.

Akai and Sato (1973) suggested that oenocytoids are perhaps involved in protein synthesis. It is also possible that they may be involved in the storage and transport of some metabolites. The functional aspects of these cells also need further investigations. Joshi and Lambdin (1996) reported the presence of many crystals which indicated the possible storage function of excretory products. However, the authors later concluded that since oenocytoids did not exhibit protoplasmic extensions or endocytosis, it became difficult to relate the presence of crystals as a storage function.

Haemocytes under experimental conditions

In the present investigation, four concentrations (ranging from Lc-30 to Lc-90 in case of acephate, aminocarb, and cypermethrin and Lc-20-Lc-60 of muristerone and methoprene) were separately applied topically on the last nymphal instar of *Dysdercus cingulatus* and the last larval instar of *Diacrisia obliqua*. The effect of these chemicals was studied at various time intervals as mentioned in "Materials and Methods".

Since four concentrations of each of the present insecticides (acephate, aminocarb and cypermethrin) were selected on the basis of percent mortality within 24 hrs after application, each of them was approximately equivalent to the corresponding concentration of other insecticide. For example, the highest concentration of acephate (0.006%) resulted in almost similar moralities in *Dysdercus cingulatus* as caused by the highest concentration of aminocarb and cypermethrin. In the same way, four corresponding concentrations of acephate, aminocarb and cypermethrin applied topically on 6^{th} instar larvae of *D. obliqua* resulted in approximately similar mortalities and other pharmacological symptoms in the affected larvae.

The application of various selected concentrations of acephate, aminocarb and cypermethrin on 5^{th} instar nymphs of *Dysdercus cingulatus* and 6^{th} instar larvae of *Diacrisia obliqua,* in general, caused pathological damage to all types of haemocytes. The nature and extent of damage to these haemocytes was generally concentration based and time dependent. The pathological symptoms were initiated with slight vacuolization, irregular cell membrane and formation of cytoplasmic extensions, eccentrically pushed nuclei in haemocytes following the lower concentrations. Whereas application of the higher concentrations resulted in more severe vacuolization and granulation in cytoplasm, breaking up of cell membrane at several places, discharge of cytoplasm, vacuolization and fragmentation of nucleus, clumping of cells, distortion and subsequent disintegration of a large number of cells.

The extent of damage to various haemocytes also depended on the time interval after exposure to the insecticides. In the nymphs of *Dysdercus cingulatus* and larvae of *Diacrisia obliqua* treated with lower concentrations, haemocytes exhibited

occasional vacuolization of cytoplasm at 6 hrs post-treatment which became more intense and extended to more haemocytes after 1 day. However, 3 days and 5 days post-treatment, haemocytes showed recovery from the toxic effects. Following the application of higher concentrations, the pathological conditions of haemocytes progressively increased upto 3 days, however, the recovery from the deleterious effects of insecticides set in after 5 days. The nymphs and larvae affected with the highest concentrations of respective insecticides died before moulting to next stage. The blood picture of these near-dying insects showed majority of haemocytes with intense nuclear and cytoplasmic vacuolization, cytoplasmic extension, broken cell membrane, discharge of cellular contents, abnormal staining reactions, achromophilia and distortion as well as disintegration of these cells. However, there was a fair population of haemocytes which was relatively free from abnormalities and appeared almost the same as that of the normal. Mitosis was observed even in the highly affected haemocytes.

Almost similar effects of the insecticides on haemocytes were earlier reported in *Calliptamus italicus* (Tareeva and Nanyukov 1931)), *Locusta migratoria* (Pilat 1935), *Prodenia eridania* (Yeager and Munson 1942), *Periplaneta americana* (Yeager, *et al*1942), *Leptinotarsa decemlineata* (Arvy *et al*., 1950), *Anagastra kuhniella* (Arnold 1952b) *Pediculus humanus* (Hopp 1953), *Tenebrio molitor* (Jones 1957), grass hopper, cockroach, *Spodoptera* and *Atteva* (Chattoraj and Sharma 1964) *Periplaneta americana* (Roy and Bagchi 1975) *Dysdercus cingulatus* (Zaidi and khan 1977), *Rhopalosiphum maidis* (Behura and Dash 1978) *Spilosoma obliqua* (Ahmad and khan 1987) and *Leptocorisa varicornis* and *Nepa cinerea* (Siddiqui 1990).

The exposure of insects to various chemicals may also induce histopathological symptoms in various tissues as reviewed by Hoskins (1940), Winteringham & Lewis (1959), Brown (1963) and Perry (1964). Even the sub-lethal concentrations, when applied topically, are capable of affecting the tissue morphology adversely (Khowaja 1997). After topical application, the insecticides enter the body and transported to the site of action. Two more or less opposing views have been put forward regarding the transport of insecticide from the point of entry to the target organ. One of them is more like that is generally accepted in pharmacokinetic studies of drug action in mammals.

This view suggests that the toxic compound is absorbed by diffusing through the integument and after crossing this barrier, conveyed by haemolymph to the target organ or to several other organs where it is accumulated, metabolized or excreted. This concept also applies to toxicants that are ingested and thus arrive at the target after passing through the gut wall. Patton (1961) and Reddy & Naidu (1967) believed that insecticides, topically applied on the body, route to the sight of action through the haemolymph. Burt *et al* (1971) have established that the haemolymph of cockroaches (*Periplaneta americana*) treated topically with a LD-90 dose of Diazoxon and LD-95 dose of pyrethrin-I contained sufficient insecticide to explain the observed disturbance of the neurophysiological functioning of the central nervous system. The observations of Soderlund (1979) on the dynamics of distribution and elimination of some pyrethroids in *P. americana* are consistent with the hypothesis that the internal distribution of these compounds is mediated by haemolymph transport. The haemolymph not only acts as carrier fluid for materials and insecticides but it also gets itself affected by their action (Chattoraj and Sharma 1964). Rakitin (1974) reported that haemocytes adsorbed a considerable proportion of administered radioactive insecticide, but did not explain how this affected the insect's response to the insecticide. On the contrary the other view regarding the transport of insecticide states that insecticides use the tracheae as port of entry and reach target by diffusing through the outer cuticular layers. The tracheae have direct excess to the central nervous system and because they are lined with the cuticle tissue, a continuous layer between the outside of an insect and target site is available for an insecticide to diffuse to the target without being forced to cross one or more membranous barrier. This mode of entry also known as lateral transport has been proposed by Gerolt (1970, 1972, 1975). According to Welling and Paterson (1985) the paths along which the insecticides enter the insect body and reach the target organ are still a controversial issue. The possibility of involvement of more than one channel in the transport of insecticides to the target organ can not be ruled out. However, the permeability to these chemicals may differ depending upon the nature of the cuticle as well as molecular structure of the chemicals.

In the present experiments on *D. cingulatus*, adipohaemocytes and plasmatocytes were the most susceptible cells followed by granulocytes,

prohaemocytes and oenocytoids. Whereas, in case of *D. obliqua*, spherulocytes were the most susceptible cells followed by plasmatocytes, granulocytes, coagulocytes, prohaemocytes and oenocytoids. Thus in both the insects, oenocytoids appeared to be the most resistant haemocytes to the insecticides. The present findings that acephate, aminocarb and cypermethrin alter morphology of haemocytes of *D. cingulatus* and *D. obliqua* are in agreement with those of other investigators. Furthermore, in the present investigations on 6th instar larvae of *D. obliqua*, affected with various chemicals, the histopathological symptoms in various types of haemocytes were also studied under TEM to get a better understanding of cellular damage. The abnormalities at the ultrastructural level were characterized by disruption of cellular and nuclear membrane and subsequent formation of gaps which led to the outflow of nuclear and cytoplasmic contents. Mild to intense vacuolization of cytoplasm was also observed and in some haemocytes the vacuolization of cytoplasm was so extensive that a network of vacuoles was formed. The cytoplasmic organelle viz., endoplasmic reticulum, mitochondria, Golgi complex, ribosomes and phagocytic vacuoles etc. became less distinct. The haemocytes became denser. Some haemocytes contained fractured and fragmented nucleus. The distinction between heterochromatin and euchromatin was occasionally lost. Many haemocytes lost their identity due to highly disrupted nuclear and cytoplasmic structures. Haemocytes of *D. cingulatus* seemed to show comparatively more variants of abnormalities than those of *D. obliqua* when observed under the light microscope. However, haemocytes of the latter species, when examined under TEM, demonstrated more clear picture of abnormalities. In another study, the effect of plumbagin (5-hydroxy-2methyl-1,4-nephthalenedione), extracted from *Plumbago zeylenica* on the haemocytes of *Dysdercus koenigii* (Tikku *et al* 1992) was investigated in laboratory. 2 and 5 µl 0.1% solution of extract was applied topically on to adults of *D. koenigii* and their haemocytes were examined by Scanning Electron Microscopy. All five haemocyte types (prohaemocytes, plasmatocytes, granular haemocytes, oenocytoids and adipohaemocytes) were affected within a period of 24-48 hrs. The effect began with an acute state of vacuolization of the affected cells and a gradual destruction of cellular organelles, followed by dissolution of plasma membrane and evacuation of internal organelles (such as mitochondria and endoplasmic reticulum). This phenomenon was responsible for a consistent elimination of the haemocytes from the

blood leading to lowering of the resistance of *D.koenigii* to plumbagin and subsequent mortality.

Haemocytes of 5th instar nymphs of *D. cingulatus* and 6th instar larvae of *D. obliqua* affected with lower concentrations of muristerone did not exhibit any appreciable alterations in their normal morphology 6 hrs post-application. Later, some haemocytes developed cytoplasmic and nuclear abnormalities characterized by vacuolization in adipohaemocytes and plasmatocytes. Just before moulting adipohaemocytes showed large vacuoles, whereas, higher concentrations induced vacuolization in more adipohaemocytes and plasmatocytes which was more intense. Prohaemocytes were moderately affected and exhibited nuclear vacuolization. Oenocytoids were only slightly affected. The haemocytes of *D. cingulatus* were more severely damaged compared to those of *D. obliqua,* however, agglutination of haemocytes was more frequently visible in *D. obliqua*. Compared to insecticides (acephate, aminocarb and cypermethrin), the ecdysteroid induced comparatively milder pathological symptoms in the haemocytes. Almost similar abnormalities were developed in the haemocytes of insects which were subjected to various selected concentration of methoprene. On the other hand, Ahmad and Khan (1988) in *Hieroglyphus nigrorepletus* concluded that injection of sublethal doses of triol and makisterone A caused development of pathological symptoms which were dose based. The highest dose of triol (6µg) destroyed most of the cells in 3-day-old adults and was more effective in destruction of haemocytes than makisterone A. The pathological effects of exogenously applied hormones on the haemocytes of insects have also been reported in *Manduca sexta* (Judy and Marks, 1971), *Spodoptera litura* (Nishi,1982 and Rao *et al* 1984), *Galleria mellonella* and *Tenebrio molitor* (Farks, 1984), *Dysdercus cingulatus* , *Spodoptera litura* and *Spilosoma obliqua* (Khan *et al* 1990). Judy and Marks (1971) for the first time attempted to observe the effect of ecdysterone on the haemocytes of *Manduca sexta* in vitro but only reported that the migratory activity of the spherule cells and plasmatocytes increased. Later, Nishi (1982) observed that the haemocytes of larvae as well as pupae of *Spodoptera litura* were pathologically affected following injection of different doses of β-ecdysone. Pathological symptoms were initiated with dissolution of the nucleus, cytoplasmic and nuclear vacuolization and irregularity in the shape of the cells by lower doses i.e. 0.5µg

and 1μg/larva. Furthermore, the pathological conditions increased progressively from 0.5 μg to 6 μg β-ecdysone/larva. The injection of different concentrations of ecdysterone in the larvae of penultimate and final instars of *Galleria mellonella* and *Tenebrio molitor* induced mitosis in plasmatocytes and prohaemocytes, respectively, and the highest mitotic index value was observed after 5-12 hr (Farks 1984). Rao *et al* (1984) studied the effect of ligation and ecdysone on total haemocyte counts in the tobacco caterpillar (*Spodoptera litura*). They concluded that hormonal injection caused increase in THC which was presumably due to direct or indirect stimulation of mitosis.

Ahmad and Khan (1988) observed that the injection of sublethal doses of Triol and Makisterone-A on 5th instar hoppers of *Hieroglyphus nigrorepletus* caused selective damage to all types of haemocytes. The degree and extent of damage to the haemocytes were dose dependent and Triol was more effective to destroy the haemocytes of these hoppers. Similarly, Khan *et al.* (1990) reported that the topical application of sublethal doses of β-ecdysone and Makisterone A on 4th and 5th instar nymphs of *D. cingulatus* and 5th and 6th instar larvae *D. obliqua* also damaged all types of haemocytes. Further, they concluded that these hormones in sublethal doses produced dose based pathological symptoms in all types of haemocytes of treated insects of the same generation on which the hormone was applied as well as on the subsequent generation. The pathological symptoms induced by muristerone and methoprene on *Dysdercus cingulatus* and *Diacrisia obliqua* in the present investigation were although of the similar nature as reported by Ahmad and Khan (1989) and Khan *et al.* (1990) but degree of damage to the haemocytes was not so severe even by the respective highest selected concentrations. The blood picture, of those nymphs and larvae which displayed obvious symptoms of poisoning, did not exhibit intense damage to blood cells and sometimes was even comparable to that of normal insects.

In addition to insecticides and hormone analogues, haemocytes responded to other factors also. Exposure of larvae of *Bombyx mori* to gamma radiation (15, 500,1000, 3000 and 5000 rad.) resulted in the appearance of abnormalities in all types of haemolymph cells, with the numbers of deformed cells increasing with increased dose (El-Maasarawy and Salam 1988).

The pathological condition of haemocytes of both the species i.e. *D. cingulatus* and *D. obliqua* can result due to a variety of physiological and toxicological stresses. The poison may react with the cellular contents resulting in the precipitation of cytoplasmic material thus making the cells fragile and which finally undergo destruction. Although certain kinds of pathological haemocytes may be more numerous following application of a given chemical these abnormalities are not peculiar or specific for the particular chemical used in the present study.

In the mammals also, especially human beings, toxicity of certain drugs and narcotics is well known on the cells of liver and erythrocytes, which become fragile and consequently haemoglobin pass out of the cell (Houssay *et al* 1955). Stelzer and Gordon (1984) found that permethrin, cypermethrin and allethrin inhibit the mitogenic response of lymphocytes stimulated by concanavalin-A and lipopolysaccharides. Furthermore, oral doses of deltamethrin, administered in mice, induced immuno-suppressive effect (Lukowicz-Ratajczak and Krechniak 1992).

THCs under normal condition

In insects the majority of the haemocytes rests on the surfaces of various organs of the body cavity but some cells circulate freely in the haemolymph. The number of circulating cells varies enormously during developmental stages and different physiological states (Wigglesworth, 1973). In both hemi- and holometabolous insects the THC tend to increase prior to each ecdysis, then decreases sharply at ecdysis and again increases shortly afterwards. In hemimetabolous insects the THC is highly variable during an individual state of development. But in many holometabolous insects the haemocyte population increases at a relatively constant rate during larval growth and reaches to a maximum in the prepupal period, then declines very rapidly at pupation and fall to minimum level during that stage. A slight increase in number of haemocytes occurrs at emergence of adults. However, the average THC is markedly lower in adults as compared to larval period. Similarly, in hemimetabolous insects, generally, the adults have comparatively lower THC than the nymphs.

In the normal last instar nymphs of *Dysdercus cingulatus*, THC followed increasing trend through the growing age. THC was comparatively low in the newly moulted nymphs, thereafter increased till 4th day showing a maximum (approximately 9000-9500cells/mm3) and subsequently underwent reduction just before the next moulting. Furthermore, the females had significantly higher THC than the males. This sexual difference in the THC might be due to possible role of the haemocytes in metabolism during oogenesis and yolk deposition in the oocytes of the females as earlier discussed in the females of *Schistocerca* (Arvy *et al.,* 1948). In other hemimetabolous insects such as *Locusta migratoria migratoriodes* (Webley 1951), *Periplaneta americana* (Patton and Flint 1959) and *Rhodnius prolixus* (Wigglesworth 1955, Jones 1964), during the nymphal period the THC decreased at each moult and then enhanced again during intermoult period. However, Wheeler (1962) in *P. americana* explained that an increase in blood volume at ecdysis would show a decrease in the number of circulating haemocytes per cubic millimeter of haemolymph. Feir and O'Connor (1969) noticed that THC increased slightly during the first three days after ecdysis (35500/mm^3 to 40500/mm^3) in *Oncopeltus fasciatus*. At this time, cell population decreased during the next three days to 26000/mm^3 and then increased to 40,000/mm^3. Bahadur and Pathak (1971) observed variations in the THC in relation to ecdysis, development and sex in the bug, *Halys dentata*. Zaidi and Khan (1975) studied THC of *Dysdercus cingulatus* and reported a peak during the intermoult period and prior to metamorphosis in the 5th instar nymphs. Furthermore, in the newly emerged females, THCs were significantly higher than those of the males of the corresponding age. Bhargava *et al.*, (1980) in *D. cingulatus* confirmed higher THC in the females than the males. Moreover, they also verified the earlier observations that in both sexes there was a gradual rise in count from the beginning of the each stage until a sharp fall before the next moult. But the number of haemocytes generally increased gradually from 3rd instar nymphs to the mature adults. The THC in adult *Chrotogonous trachypterus* and *Acrida exaltata* varied from 1110 to 3020 and 1900 to 3326 respectively (Sharma and Gupta 1979). In their studies on THCs in Orthoptera, Odonata, Hemiptera and Homoptera, Tauber and Yeager (1935) found that the average count from the females was higher than the males. The high female counts at times appeared to be associated with the period of oviposition. Lee (1961) in *Schistocerca gregaria* suggested that the decrease in THC at the moult was due to an

increase in blood volume. However, Shapiro (1979) concluded that the relationship between blood volume and THC was not clear-cut. Jones (1956) and Arnold (1966) felt that decreases in THC were attributable to the adherence of haemocytes to tissue surfaces.

In the present study, the 6th instar larvae of *Diacrisia obliqua* exhibited an increase in THC till 4th day and thereafter showed reduction till the next moulting. In two-day-old pupae THC was significantly low. Variations in THCs were observed in normal as well as in different experimental conditions in *Galleria mellonella* (Stephens 1963; Srivastava and Richards 1964; Shapiro 1966; Jones 1967 and Jones and Liu 1968 and 1969). The THC of *Galleria mellonella* increased during larval development in both heat fixed and unfixed larvae but counts from the heat fixed insects were higher than those of the unfixed larvae. In the 5th instar larvae of *Pseudoletia unipunctata*, the THC was high at the time of moulting. Thereafter, the cell population decreased and continued to fall until pre-pupation (Wittig 1965). Shapiro *et al,* (1969) reported insignificant change in THC in *Heliothis zea* larvae from 6-10 days. Kitano (1969) observed higher THC in early 5th instar larvae than those in late 5th instar of *Pieris rapae crucivora* females. Raina and Bell (1974) studied THC of diapausing and non-diapausing larvae of *Pectinophora gossypiella* and found that during diapause there was a significant reduction in THC but on termination of diapause, the THC increased. Arnold and Hinks (1976) reported that, in general, cell number increase from 6000/mm^3 to 20000/mm^3 in 2nd to 6th instar larvae of *Euxoa declarata*. In *Bombyx mori* there was a gradual increase in THC from 1st to 3rd instar larvae and thereafter a remarkable increase occurred from 4th to 5th instar. Kaaya and Otien (1981), in *Glossina morsitans* and *G. Pallidipes* found that THC dropped significantly during the first 48 hrs following emergence and then leveled off with minor fluctuations. The sudden drop in THC was resulted due to remarkable decrease in the number of the spindle cells in the haemolymph. Nishi (1982) reported gradual increase in THC from 5th instar to late 6th instar larvae (pre-pupae) of *Spodoptera litura* followed by reduction in pupae.

THCs under experimental conditions

In the present investigations the THC of treated 5th instar nymphs of Dysdercus *cingulatus* with acephate, aminocarb and cypermethrin was determined at various time intervals after the application. The lowest concentrations of the respective insecticides (0.001% acephate, 0.0025% aminocarb and 0.0004% cypermethrin) generally enhanced THC slightly after 6 and 24 hrs as compared to the corresponding controls. Whereas, after 3 days onwards, there was reduction in the total counts. On the other hand, the highest concentrations of acephate (0.006%), aminocarb (0.008%) and cypermethrin (0.001%) significantly reduced the THCs by 43.55%, 58.28%, 58.26% after 6 hrs, by 50.10%, 74.75%, 61.99% after one days, by 67.41%, 81.68%, 70.79% after 3 days and by 67.54%, 77.51% and 66.97% after 5 days of treatment as compared to the corresponding controls. The application of remaining two concentrations, of each of the three insecticides (0.002% and 0.004% acephate, 0.004% and 0.007% aminocarb, as well as 0.0006% and 0.0008% cypermethrin) falling between the lowest and the highest ones induced reduction in the mean THCs at various time intervals which, however, was statistically insignificant.

Similarly, the application of the lowest concentration of acephate, aminocarb and cypermethrin (0.04%, 0.1% and 0.0025% respectively) on the last larval instar of *Diacrisia obliqua* (2 day old) generally enhanced the population of circulating haemocytes at 6hrs, 1 day, 3 days and 5 days post treatment. The next higher concentrations of respective insecticides (0.08%, 0.2% and 0.005%) elicited a mixed response. In most of the cases the haemocyte pupulation/mm^3 of haemolymph increased while in some, it showed a decline.

The next higher concentration (corresponding to Lc-70 of respective insecticides) of the above mentioned insecticides (0.1%, 0.4% and 0.01%) induced a reduction in haemocyte population as compared to the respective controls. Furthermore, application of the highest concentration of acephate, aminocarb and cypermethrin (0.2%, 0.6% and 0.15% respectively) caused statistically significant reduction in haemocyte population at various time intervals. When the treated larvae moulted to next stage i.e. pupal stage, the lower concentrations induced a positive

response in haemocytes, whereas, the higher concentrations had a negative effect on the haemocyte population. However, the changes were statistically insignificant.

A vast amount of data has been accumulated on the changes in haemocyte population but much of the work offers only a glimpse of haemocyte changes (Arnold 1974). However, only a few observations are available on the effect of poisons on THC. Some authors have recorded an increase in total counts, whereas, others observed reduction. Increase in THC occurred in chloroform fed *Anagasta* larvae (Arnold 1952 b). Jones and Tauber (1954) observed a significant increase in THCs of last-instar *Tenebrio* larvae following fumigation with nicotine. Gupta and Sutherland (1968) observed and increase in THC in chlordane treated *Periplaneta americana* THC increased. Arvy *et al.*, (1950) also noted an increase in THC in *Chrysomela decemlineata*. Basu and Chaudhury (1975) also reported increase in THC whem *P. americana* was treated with malathion and benzene hexachloride. They pointed out that increase takes place because of mitotic division in haemocytes. Shukla and Bahadur (1986) in *Poecilocerus pictus* treated with dichlorvos and phosphamidon, reported an increase in THC 2 days following treatment which was comparatively more in dichlorvos treated male insects. These authors could not observe mitotically dividing cells and regarded the increase as the outcome of detoxification of poison.

On the other hand, the reduction in THCs of treated insects was registered in some cases. Both dichloroethyl ether and methyl bromide caused a significant decrease in THC. Pilat (1935) also observed the reduction in THCs in *Locusta migratoria* by Fisher (1936) in *Blatta orientalis* and by Trehan and Pajni (1961). Shukla and Bahadur (1986) reported a fall in THC in *Poecilocerus pictus* in first two days after treatment with dichlorvos and phosphamidon. On the other hand, Jones (1957) showed that THCs in unfixed and heat-fixed *Tenebrio* larvae treated with pure, finely powdered p,p'- DDT were within the normal range prior to moribund stage. Moreover, when *Tenebrio* larvae were exposed to sodium fluoride dust, none died or showed signs of detoxification. Neither THC nor DHC showed any changes from the "norm" and abnormal haemocytes were not observed (Jones and Tauber 1954). On the basis of these studies they felt that the haemocytes played a very small role, if any, in the defense of the insect.

Besides insecticides, changes were found to occur in THC in response to the treatment with exogenously applied insect hormone analogues. In the present investigations the application of lower concentrations of the selected ecdysteroid (i.e. muristerone) as well as juvenoid, (methoprene) on the 5th instar nymphs of *D. cingulatus* and the 6th instar larvae of *D. obliqua* resulted, in general, in the increase in total count which, however, was statistically insignificant at 5% level. Whereas, the higher concentrations caused statistically insignificant reduction in THCs. Compared to insecticidal treatment, the application of the present ecdysteroid and juvenoid did not induce drastic fall in THC even by the higher concentrations.

Earlier, Ahmad (1986) found a significant reduction in THC in the 5th instar hoppers of *Hieroglyphus nigrorepletus* following injection of even sublethal doses of Makisteron A (a phytoecdysteroid). She found that THCs were more affected in the females than in the male hoppers. Nishi (1982) observed the effect of different doses (0.5, 1.0, 2.0, 4.0 and 6.0 μg) of β-ecdysone (moulting hormone) on the THC of the 6th instar larvae, prepupae and pupae of *Spodoptera litura*. According to her, THCs were enhanced with respect to 0.5 μg and 1 μg doses in the 6th instar larvae and the prepupae moulted from the treated 6th instar larvae but such an increase was statistically insignificant as compared to control. Although similar trend of increase was also observed in the present investigation but the concentrations of the ecdysteroid (muristerone) were comparatively higher and the response of haemocytes was most pronounced in the same instar on which application was made and the effect became somewhat diluted when the treated insects moulted to the next stage. Again, in *S. litura* Rao *et. al.* (1984) observed the effect of ligation and ecdysone on THC. Ligation of 5th instar larvae behind the thorax resulted initially in a decrease in THC in both the posterior and the anterior halves. But 48 hrs after ligation the cell number increased in the anterior half only. They speculated that increase was due to the presence of prothracic glands and haemopoetic organs in the anterior part of the body. Ahmad (1986) further examined THC in the larvae of *Spodoptera litura* following ingestion of different doses of β-ecdysone (ranging from 0.5 to 6.0 μg) by the 5th and 6th instar larvae. In the 6th instar larvae moulted from treated 5th instar, the THC showed significant reduction. Similarly, in the prepupae and pupae moulted from treated 6th instar larvae, THC was also reduced significantly. Whereas, in the present

investigation on *D. cingulatus* and *D. obliqua* such drastic reduction in total cell number in 1 mm^3 of blood was not observed even in the insects otherwise showing severe pharmacological symptoms due to action of hormone or insecticide. Pathak (1991) studied the effects of endocrine extract on the blood volume and population of haemocytes in *Halys dentata* (Pentatomidae: Heteroptera). Diuretic hormone present in the extract of brain and corpora cardiaca resulted in an increase in the THC. Antidiuretic hormone present in the thoracico-abdominal ganglion of unfed adults increased blood volume, which caused a significant decrease in the THC. Extract from the corpora allata (juvenile hormone) which had no effect on blood volume influenced the THC by mobilizing non-circulating sessile haemocytes.

In addition to the insecticides, some workers studied the effect of injection of certain non-particulate as well as particulate matter and solutions and recorded changes in the haemocyte population. After injection of saline, the THC increased in *Euproctis* (Paillot 1923) and in *Schistocerca* (Lee 1961), but was reduced in *Galleria* (Jones 1962). THCs were reduced in *Prodenia* following injection of Trypan blue (Rosenberger and Jones 1960). The THC was increased in *Rhodnius* (Wigglesworth 1956) but was reduced in *Galleria* (Werner and Jones 1969) following injection of ink. Moreover, injection of starch resulted in increase in THC; injection of sheep erythrocytes led to a decrease (Werner and Jones 1969). Takada and Kitano (1971) reported reduction in THC in first 24 hrs and then an increase during next 48 hrs following injection of India ink to *Pieris rapae crucivora*. Wittig (1966) in *Galleria* observed that injection of low doses (6.6 million - 13.3 million particles) of latex particles resulted in an increase in THC, whereas, high doses (66.3 million particles) resulted in decrease in THC. When third instar larvae of *Tipula paludosa* (Green and Carter, 1991) were exposed to (i) ethyl ether vapours for 20 min.(ii) acetic acid vapours for 10 min. or (iii) ethyl ether for 20 min. followed by acetic acid vapours for 10 min, then in the first case there was 1.6 fold increase in THC, in second case no significant increase and in the third case 2.8 fold increase was observed.

Another important factor that causes changes in THC is starvation but the data are contradictory. Jones (1950) carried out a series of experiments on the effects of starvation on the haemocytes of last-instar larvae of *Tenebrio*. Larvae starved for

periods ranging from 1 to 30 days showed THCs within the normal range. In 1952, Jones and Tauber noted decrease in THCs after prolonged starvation. In both, *Leptinotarsa* (Arvy *et al.*, 1948) and *Prodenia* (Rosenberger and Jones 1960), THC increased during starvation. The authors in both cases suggested that changes in blood volume were perhaps responsible for the increase in THC. Nittono (1960) reported that the THC in starved silkworm larvae decreased gradually and became lower than the control. In *Galleria*, THC among control insects increased, whereas, that of starved insects decreased (Shapiro, 1966). Feeding and 20-hydroxyecdysone treatment reversed the effect of starvation on THC (i.e. increased the THC) in larval form of *Corcyra cephalonica* (Sujatha *et al.*, 1991)

In the present investigation, it has been observed that application of acephate, aminocarb and cypermethrin as well as muristerone and methoprene affects the feeding of the treated nymphs and larvae. The effect on feeding was, however, concentration based. Thus the changes in THC in present observations may be associated, in part, with the feeding abnormalities caused by the application of chemicals. The physiological state of individual insect of same age and stage also causes variations in the THCs which, in the present study, is reflected by high standard deviation even in the untreated and solvent treated insects.

DHCs under normal conditions

In the present investigation, DHC of normal 5^{th} instar nymphs, adult males and females of *Dysdercus cingulatus* as well as 6^{th} instar larvae and pupae *Diacrisia obliqua* were determined. In the last nymphal instar of *D. cingulatus* the proportion of prohaemocytes increased gradually from newly ecdysed nymphs till 4^{th} day post-ecdysis when their peak occurred. Thereafter their population decreased drastically before the next moulting. Subsequently, in the newly emerged males and females it was proportionately low. In the adults of the same species, Zaidi and Khan (1975) stated that the percentage of prohaemocytes was very small in comparison to that of the plasmatocytes and adipohaemocytes. Jones and Liu (1961) observed that in *R. prolixus* prohaemocytes decreased and adipohaemocytes increased prior to the next

ecdysis. In contrast to the present observation on *D. cingulatus*, in *P. americana* (Roy and Bagchi 1973) the percentage of prohaemocyte was highest compared to other haemocytes. In *Chrotogonus trachypterus*, the prohaemocytes ranged from 26-32% of the total free haemocytes in circulation, showing fairly high percentage (Sharma and Dutta 1979). In *Leptocorisa varicornis*, after 3rd moult, the percentage of prohaemocytes subsequently increased, which also showed an increase up to 2 days in the adult males and females following the moult (Siddiqui 1990). However, in 3rd instar *Nepa cinerea* the prohaemocyte population decreased up to 2 days and the opposite situation occurred from 3rd to 6th day. Similarly, in the males the females too, the proportion of these cells showed reduction up to 3 days after ecdysis (Siddiqui 1990). This trend in *N. cinerea* is somewhat similar to that of *D. cingulatus* after two days following ecdysis.

In the present investigation on *D. cingulatus* the plasmatocyte proportion did not show much variation throughout the last nymphal instar though it underwent a progressive decline as the age of the nymphs advanced and the population of these cells reduced to the lowest on the 4th day post moult. In the adult females the plasmatocytes were proportionately more than those in the adult males and constituted about half of the total cell population. In the same species, Zaidi and Khan (1975) also reported that the percentage of plasmatocytes was higher in the newly emerged females than in the males of the corresponding age. However, in both sexes, plasmatocytes percentage increased with aging especially after the first reproductive cycle. In *Acheta domesticus* the number of plasmatocyte went down in the last moulting cycle (Hrdy 1959). Likewise in *R. prolixus* there was a decrease in population of these cells at the time of moulting (Jones and Liu 1961). Thus the trend of plasmatocytes population was like that of the present observation on *D. cingulatus*. In *Periplaneta americana* plasmatocyte population was comparatively lower than prohaemocytes (Roy and Bagchi 1973). In *Chrotogonus trachypterus*, plasmatocytes constituted majority of the free haemocytes (Sharma and Dutta 1979). In *Leptocorisa varicornis*, after the third moult the percentage of plasmatocytes decreased slowly but significantly before moulting like that of *D. cingulatus*. Whereas, in adult males and females plasmatocyte population increased. Consequently, plasmatocytes constituted the highest population of free haemocytes in the adults of this species (Siddiqui 1990).

Similar trend was found in the 3rd instar nymphs, adult males and females of *Nepa cinerea* (Siddiqui 1990). The reduction in percentage of prohaemocytes and increase in the plasmatocytes suggested the transformation of prohaemocytes into plasmatocytes. But in the present investigations on *D. cingulatus* the changes in prohaemocytes and plasmatocytes cannot be justified on the basis of transformation of prohaemocytes into plasmatocytes because the trend is not significant.

In the *Dysdercus cingulatus* the most remarkable change occurred in the population of adipohaemocytes. Their proportion showed a gradual increase throughout the last nymphal instar and reached to the maximum just before the next moult. Moreover, in one-day-old adult males their number was significantly more than the previous stage as well as that of females. The reduction in plasmatocyte population simultaneously with increase in adipohaemocytes appears to indicate the possible transformation of plasmatocytes to adipohaemocytes. Earlier, Zaidi and Khan (1975) in the same species recorded almost the similar trend. They further elaborated that adipohaemocyte population decreased with advancing age of the adults and became low in both sexes especially after the first reproductive cycle. In *Rhodnius prolixus* these haemocytes increased prior to ecdysis (Jones and Liu 1961), however, at the time of moulting their population underwent a reduction. On the other hand, in *Leptocorisa varicornis* (Siddiqui 1990), the adipohaemocytes population decreased slowly but significantly before moulting. However, following emergence of adults their population dropped in two-day-old males and the females. In *Nepa cinerea* (Siddiqui 1990) the population of these cells increased during intermoult period.

In the present investigations on *D. cingulatus* the granulocytes population showed an inconsistent change, whereas, the oenocytoid population was fairly constant throughout the last nymphal instar. In *Rhodnius prolixus* (Jones and Liu 1961) the granulocytes increased at the time of moulting, however, after ecdysis granulocytes were less in number compared to plasmatocytes and oenocytoids. Zaidi and Khan (1975) reported that granulocytes and oenocytoids were poorly represented in adult *D. cingulatus*. In *Leptocorisa varicornis*, after the third moult the percentage of granulocytes decreased slowly but significantly before the next moulting and that of oenocytoids subsequently increased. Furthermore, following the emergence of males

the population of oenocytoids underwent reduction, whereas, that of granulocytes showed no change. In the females oenocytoid and granulocyte percentage dropped. In the 3rd instar *Nepa cinerea* granulocytes increased whilst oenocytoids decreased upto two days after moulting and the opposite occurred from third to sixth day after moulting. In males and females of *N. cinerea* granulocytes increased and oenocytoids decreased upto three days after ecdysis. Decrease in the percentage of prohaemocytes and increase in that of plasmatocytes suggested the transformation of prohaemocytes into plasmatocytes (Siddiqui 1990). Increase in the percentage of plasmatocytes and granulocytes contribute the stronger defensive abilities of adults (Hazarika and Gupta 1987).

In the present observations on the normal final larval instar of *Diacrisia obliqua*, prohaemocytes and plasmatocytes were found to be main haemocyte types and these together constituted approximately 75-80% of total haemocytes. Other haemocytes viz. granulocytes, spherulocytes, oenocytoids and coagulocytes combined together, made up the remaining 20% to 25% of the total blood cells. On the other hand, in one-day-old pupae, spherulocytes and oenocytoids were altogether absent, whereas, plasmatocytes were predominantly present.

In *D. obliqua* the population of prohaemocytes underwent a progressive decline as age of 6th instar larva increased from 1 day to 6 days (prohaemocyte population: 33.96% at 1st day, 32.08% at 2nd day, 25.73% at 4th day and 24.86% at 6th day). Moreover, in one-day-old pupae it was the lowest (16.51%). In *Galleria* (Shapiro 1966) and in *Prodenia* (Yeager 1945) a peak in population of prohaemocytes occurred in the middle of the penultimate instar. The proportion of prohaemocytes then decreased and subsequently increased as the last instar larvae approached prepupation. According to Nittono (1960), in the silk worm, peak of prohaemocyte population occurred 24 hr after each moult. The percentage of prohaemocytes in *Euxoa* reached a maximum during the third instar and thereafter decreased (Arnold and Hinks 1976). In *Galleria*, *Prodenia* and *Bombyx* reduction in prohaemocytes occurred during the last larval instar upto the prepupation, when an increase occurred. Nishi (1982) in *Spodoptera litura* found maximum prohaemocyte percentage in the pupal stage.

In the present study on *D. obliqua*, the plasmatocyte population did not vary much throughout the last larval instar (plasmatocyte population: 50.66% at 1st day, 50.72% at 2nd day, 53.10% at 4th day and 48.20% at 6th day), whereas, in pupae their population showed a remarkable increase (71.21%). In the larvae of *Heliothis virescens* plasmatocytes decreased from day 5 to day 8 and then increased upto pupation (Vinson 1971). Whereas, in *Pseudoletia unipunctata*, plasmatocytes increased during 6th instar and during pupation (Wittig 1965). Nishi (1982), in *Spodoptera litura*, recorded that the percentage of plasmatocytes increased from 5th instar to late 6th instar larvae and thereafter declined in 2 day old pupae

In the present study the granulocyte population of 6th instar larvae of *Diacrisia obliqua* increased from 2.94% in one day old to 12.16% in 6-day-old insects. Thereafter, in one-day-old pupae it again declined (3.57%). Yeager (1945) in *Prodenia eridania* also reported an increase in granulocytes up to the middle of the last instar. In this insect the proportion of granulocytes then decreased, whereas, in *Galleria* a plateau was reached (Shapiro 1966) and in *Bombyx mori* the proportion of granulocytes increased during the last instar (Nittono 1960). In the larvae of *Euxoa* these cells occurred in greater proportions than any other haemocyte type. These cells decreased from 40% to 31% in second instar larvae and then increased during larval development.

Adipohaemocytes, in the present study, were observed only in the nymphs and adults of *D. cingulatus*, whereas, in the last instar larvae and pupae these haemocytes were absent. In both *Anagasta* (Arnold 1952 a) and *Prodenia* (Yeager 1945) the highest percentage of adipohaemocytes occurred in pupae. In *Galleria a* spectacular increase in the proportion of adipohaemocytes from 0 to 69% occurs when larvae begin to spin cocoons (Jones 1964). In a subsequent study (Jones 1967a), adipohaemocytes were initially observed in 17 day old larvae. In general the proportion of these cells increased during cocoon formation. Shapiro (1966) observed an increase in adipohaemocytes during larval development. In *Hyalophora cecropia* adipohaemocytes included diverse cells. Even though subclasses were often indistinguishable when fixed and stained, most cells were easily categorized under phase-contrast microscope.

The proportion of spherulocytes slightly increased from 1.72% in one-day-old larvae to 3.58% in 6-day-old 6th instar larvae of *D. obliqua*. These haemocytes were absent in pupae. The oenocytoid and coagulocyte population was fairly constant throughout the last larval instar of *D. obliqua*. In *Galleria*, spherulocytes population decreased as the age of 6th instar larvae increase. The sperulocytes further decreased in number during prepupation and were not oberved in pupae. Population of oenocytoids and coagulocytes did not vary much in 6th instar larvae, prepupae and pupae. Trends similar to those obtained in *Galleria* (Shapiro 1966, Jones (1967) were found in *Sarcophaga* (Jones, 1956), *Bombyx* (Nittono, 1960) and *Pseudoletia* (Wittig 1965) for spherulocytes, in *Prodenia* (Yeager, 1945) *Tenebrio* (Jones 1950), *Bombyx* (Nittono 1960) and *Euxoa* (Arnold and Hinks 1976) for oenocytoids, and in *Prodenia* (Yeager 1945) for podocytes. The percentage of spherulocytes was higher in 6th instar larvae than that of 2-day-old 5th instar and late 6th instar larvae. Bardoloi and Hazarika (1995) reported marked changes during the different larval instars of *Antheraea assama*

Changes in DHCs, as reported by various investigators, from the viewpoint of their postembryonic development explicitly explain the increase in the percentage of prohaemocytes due to their production. Moreover, the reduction in prohaemocytes and enhancement in plasmatocytes was due the transformation of prohaemocytes into plasmatocytes (Crossley 1975, Shapiro 1979). Increase in the percentage of granulocytes, and spherulocytes in the present species (*D. obliqua*), reduction in prohaemocytes and/or plasmatocytes are in compliance with the conversion of plasmatocytes into granulocytes, and spherulocytes. However, since prohaemocytes and plasmatocytes both declined in last larval instar of *D. obliqua*, transformation of prohaemocytes into plasmatocytes at this stage seems unlikely. Moreover, enhancement of plasmatocytes proportion in pupal stage may be accounted for the increased phagocytic activity to facilitate the clearance of debris of lysed larval tissue from the haemolymph of pupa.

DHCs under experimental conditions

The application of various concentrations (0.001, 0.002, 0.004 and 0.006%) of acephate on 5th instar nymphs of *Dysdercus cingulatus* caused a significant increase in population of prohaemocyte, oenocytoid and disintegrating cells 6 hrs, 1 day, 3 days and 5 days post-treatment. Whereas, population of plasmatocytes and adipohaemocytes was decreased significantly. Adipohaemocytes were completely absent in smears on 3 and 5 days post-treatment with higher concentrations (i.e. 0.004 and 0.006%). The granulocyte population showed inconsistent variation following application of lower concentrations, however, the highest concentration (0.006%) caused a reduction up to three days and after that completely destroyed these haemocytes. On the other hand, in successfully emerged adult males and females the population of various haemocytes showed insignificant variation as compared to control, though, the prohaemocytes were considerably high in number as compare to other cells. Treatment with various concentrations (0.0025, 0.004, 0.007, 0.008%) of aminocarb caused a significant reduction in the population of plasmatocytes, granulocytes and adipohaemocytes throughout the last nymphal instar. The adipohaemocytes and granulocytes were completely destroyed after 1 day following the treatment with the highest concentration (0.008%). Prohaemocyte percentage showed insignificant and inconsistent changes up to 3 days but after 5 days showed significant enhancement with higher concentrations. Similarly, oenocytoids, too, were significantly high in proportion since these cells were not affected much even by the highest concentration. The population of disintegrating cells was consistently high throughout the last nymphal instar. In adult males and females prohaemocyte population was significantly high. Almost similar trend of alterations in differential haemocyte counts was observed in the nymphs and adults of *Dysdercus cingulatus* treated with various concentrations of cypermethrin.

When the treatment of various selected concentrations (0.04, 0.08, 0.1 and 0.2%) of acephate was made on the last larval instar of *Diacrisia obliqua*, plasmatocytes showed significant reduction and disintegrating cells exhibited significant increase throughout this instar, whereas, other haemocytes exhibited inconsistent alterations in population. Spherulocytes were completely absent in

majority of smears observed. Granulocytes, in general, showed a reduction in population, which was concentration based. In the successfully formed pupae, these changes in haemocyte population were insignificant compared to control. In the 6th instar larvae of *Diacrisia obliqua* treated with various concentrations of aminocarb (0.1, 0.2, 0.4, and 0.6%), the population of plasmatocytes was initially enhanced after 6 hrs in response to lower concentrations, however, the highest concentration (0.6%) significantly reduced their population. Changes in prohaemocyte population were highly inconsistent throughout this larval instar. Spherulocytes, granulocytes and coagulocytes were mostly destroyed by higher concentrations. Similarly, the disintegrating cells were significantly high in the smears of haemolymph of the larvae affected with higher concentrations of aminocarb. In the pupal stage also the disintegrating haemocytes were proportionately higher. Similarly, following the application of various concentrations of cypermethrin (0.0025, 0.005, 0.01, 0.015%), the plasmatocytes were principal cells which underwent excessive cytoplasmic and nuclear disintegration and thereby subsequent reduction in population. Prohaemocytes mostly remained in the normal range, however, sometimes exhibited significant increase in population. Spherulocytes and granulocytes were proportionately fewer by the lower concentrations but were completely absent in the blood smears of larvae affected with higher concentrations. The population of disintegrating cells was consistently high at the respective intervals after treatment.

Following exposure (ingestion) of *Periplaneta americana* to chlordane (a chlorinated hydrocarbon), plasmatocytes, granulocytes, spherulocytes and coagulocytes increased in percentages (Gupta and Sutherland 1968). They applied a different chemical (i.e. a chlorinated hydrocarbon) than the group of acephate, aminocarb and cypermethrin, and the applied concentrations of chlordane were only sublethal whereas, in the present observations on *D. cingulatus* and *D. obliqua* the applied concentrations of acephate, aminocarb and cypermethrin had a vide range i.e. Lc-30 to Lc-90, hence the present observations can not be compared with that of Gupta and Sutherland on *P. americana*. In the present case plasmatocytes generally decreased by the application of all the concentrations of respective chemicals. However, following fumigation with dichloroethyl ether, carbon tetra chloride and methyl bromide, the proportion of prohaemocytes and oenocytoids did not change

greatly following fumigation but the plasmatocytes/adipohaemocytes relationship was markedly changed (Arnold 1952 b). These changes indicated the stage of starvation. Arnold felt that adipohaemocytes were important in the recovery of the insect because of their ability to synthesize fats and contribute to the insect's energy pool. Toumanoff and Lapied (1950) noted increase in the number of prohaemocytes and granulocytes in *Galleria*. Arvy *et. al.* (1950) and Jones (1957) observed increase in these cells in *Chrysomela* and *Tenebrio*, respectively. However, when *Tenebrio* larvae were exposed to sodium fluoride dust, DHC did not show any change from the norm and abnormal haemocytes were not observed (Jones 1957). Arnold (1952 b) reported that the response of *Anagasta* haemocytes went through three stages (i) passive - active transformation (ii) pathological changes (iii) regenerative changes, resulting in an increased mitotic index and an increase in cell number, especially the adipohaemocytes. Rotenone (Tauber 1935) and nicotine and sodium fluoride (Jones and Tauber, 1954) had no effect on the number of mitotically dividing cells in *Blatta* and *Tenebrio*, respectively. In *Anagasta* larvae, changes in mitotic index were noted (Arnold 1952 b). The foregoing results on the effect of chemicals on the haemocytes of various species as mentioned in the last paragraph are based on the effect of mostly chlorinated hydrocarbons or otherwise different chemicals (fumigants and insecticides of plant origin. Therefore, the present data on *D. cingulatus* and *D. obliqua* with regard to the effect of insecticides of three different groups i.e. organophosphate, carbamate and pyrethroid represented by acephate, aminocarb and cypermethrin respectively on THC and DHC reveal the haemocyte picture of the affected nymphal, larval, pupal and newly emerged adults which can not be compared with information available from earlier studies in this regard. Furthermore, the present data on changes in haemocyte population (THC and DHC) of *D. cingulatus* and *D. obliqua* affected with the present insecticides are original and comparative which may provide information to future investigators on insect haemocytes.

Besides insecticides, other substance viz. inert material, particulate matter, solutions etc. when administered into insects, caused alterations in differential counts. Following injection of India ink, granulocytes increased in percentage and plasmatocytes decreased. In *Pieris rapae crucivora* (Takada and Kitano 1971) the phagocytic index increased from 26% (2 hrs) to 75% (72hrs) during the experiment

period. During this time, granulocytes increased to represent 92% of all circulating haemocytes. About 69% of all granulocytes and 33% of all plasmatocytes were found to be phagocytic. When latex particles were injected, plasmatocytes were the primary phagocytic haemocytes, but granulocytes were also phagocytic (Takada and Kitano 1971). Wittig (1966) observed an increase in granulocytes and a decrease in plasmatocytes following injection of Chinese ink in *Galleria*. Following injection of India ink, plasmatocytes and adipohaemocytes increased; and the proportion of spherulocytes, oenocytoids and mitotically dividing haemocytes did not change (Werner and Jones 1969). Moreover, the percentage of adipohaemocytes increased in *Galleria* after injection of starch or sheep erythrocytes. No change was observed in spherulocytes, oenocytoids and mitotically diving cells in either case (Werner and Jones 1969). However, following the injection of low doses of latex particles proportions of granulocytes and plasmatocytes were not altered, whereas, at higher doses, the granulocytes increased and the plasmatocytes decreased (Wittig 1966). Following injection of the distilled water into *Tenebrio* larvae, the percentage of fusiform cells and prohaemocytes reduced (Jones 1950). In *Pseudoletia* larvae (Wittig 1966), the DHC on the other hand, did not change following the injection of water. Injection of sterile salt solutions in *Tenebrio* larvae induced increase in prohaemocyte and plasmatocyte percentage and a reduction in adipohaemocyte. In these larvae, the greatest increase in prohaemocytes was occurred 24-hrs post injection; no increase in this cell type occurred in adults (Jones 1950).

The above paragraph explains the changes in different haemocyte population by administrating inert particles as well as exposure to certain pathogens in different insect species. The data presents inconsistency with regard to changes in population of different haemocytes of various insects. Here again the present record on *D. cingulatus* and *D. obliqua* on haemocytes of affected nymphs, larvae, pupae and adults cannot provide any parallelism. Therefore the data given in the paragraph may be regarded as only the information.

Besides these, starvation also affected the DHC in experimental insects. *Tenebrio* larvae, starved for periods ranging from 1-30 days showed increase in granulocytes and decrease in plasmatocytes. After 120 days of starvation, there was

an increase in prohaemocytes and a marked apparent haemocytopenia. In 1952, Jones and Tauber noted decrease in the relative proportion of plasmatocytes after prolonged starvation. Among fourth instar *Anagasta* larvae starved for 24 hrs. Arnold (1952 a) found significant increase in relative numbers of adipohaemocytes but a decrease in the number of fusiform plasmatocytes. In *Bombyx* larvae, the relative proportion of spherulocytes increased as the starvation period increased and sometimes these cells accounted for more than 50% of the total haemocyte population. In most of the spherulocytes there was a decrease in the number of spherules but increase in the size of each spherule (Nittono 1960). Significantly more round plasmatocytes were found in starved *Galleria* larvae during the first three days. Even though the percentage of granulocytes increased in starved as well as control groups, a small number of these haemocytes occurred in starved insects owing to the significant decrease in THC. Other changes that occurred were (1) decrease in adipohaemocytes, spherulocytes and cells in mitosis in starved insects (2) increase in degenerating cells in starved insects. The present information on changes in the population of different haemocytes of nymphal and adult *D. cingulatus* and larval as well as pupal *D. obliqua* also forms a part of the effect of starvation consequently caused by the anti-feeding effect of the respective insecticide concentration applied on these insects.

In the present investigations, besides insecticides, effects of an ecdysteroid and a juvenoid were also observed on the DHC of 5^{th} instar nymphs of *Dysdercus cingulatus* and 6^{th} instar larvae and pupae of *Diacrisia obliqua*. When different concentrations of muristerone (0.2, 0.4, 0.6 and 0.8%) were applied on one day old 5^{th} instar nymphs of *Dysdercus cingulatus*, the adipohaemocytes increased in population with stronger concentrations except the highest one which caused a reduction in the number of these cells. The population of damaged cells also enhanced with the increasing concentrations but their proportions were comparatively more in the insects affected with acephate, aminocarb and cypermethrin. Prohaemocyte population showed slight increase 5 days post-treatment. Granulocytes underwent reduction in percentage. Oenocytoids and plasmatocytes were comparatively unaffected. However, after moulting to adult stage, the prohaemocytes population was significantly higher than before. The nymphal-adult intermediates showed plasmatocytes as the

predominant type of haemocytes. The population of damaged cells was also quite high in these insects.

When various concentrations of methoprene (0.04, 0.08, 0.1 and 0.2%) were applied on 5th instar nymphs of *D. cingulatus*, adipohaemocytes and granulocytes dropped in number significantly throughout nymphal life. Other haemocytes exhibited changes in a narrow range, which were insignificant in comparison to control. The population of damaged cells was significantly high in the nymphs affected with 2 higher concentrations (0.1 and 0.2%). In adult males, the prohaemocytes were significantly high, whereas, in the females although there was an increase in their population, it was not statistically significant. Moreover, the damaged cells were significantly more in adult males and in females. The nymphal-adult intermediates contained high percentage of adipohaemocytes as compared to those, which formed by the application of muristerone.

After six hrs following application of various concentrations of muristerone on 6th instar larvae of *D. obliqua* the populations of various haemocytes were insignificantly affected. But the percentage of disintegrating cells was significantly high. After one day, prohaemocyte percentage was enhanced slightly, the granulocytes increased significantly whereas plasmatocytes proportion was reduced. After three days of application, plasmatocytes were significantly reduced and disintegrating cells were significantly enhanced. Whereas, spherulocytes were absent in the smears of larvae affected with higher concentrations. In successfully formed pupae the prohaemocyte population was slightly enhanced and that of plasmatocytes showed a declining trend with increasing concentrations of muristerone.

Likewise, when different concentrations of methoprene were topically applied to 6th instar larvae of *D. obliqua*, prohaemocytes were initially declined, however, after one day their population progressively enhanced till ensuing ecdysis and showed a significant increase as compared to control. On the other hand plasmatocytes under went a consistent reduction following the application of increasing concentration of methoprene. Similarly, granulocytes and disintegrating cell population also exhibited a

significant rise throughout the 6th larval instar as well as 1-2 day old pupae as compared to control.

Nishi (1982) studied the effect of injection of different doses (0.5 μg, 1.0 μg, 4 μg and 6 μg) of β-ecdysone on DHC in *Spodoptera litura* and found that the percentage of plasmatocytes increased in 2 days old 6th instar larvae and prepupae, whereas, that of prohaemocytes enhanced in 6th instar larvae and prepupae but declined in 2 day old pupae with respect to injection of 0.5 μg and 1.0 μg doses. The percentage of adipohaemocytes, subsequently, dropped by the application of 0.5 μg and 1.0 μg doses. The percentage of adipohaemocytes subsequently dropped by the application of 0.5 μg dose in the 6th instar larvae, prepupae and pupae. The percentage of podocytes increased in 6th instar larvae but declined to negligible percent in the prepupae and pupae. The oenocytoids were the only resistant cells to B-ecdysone as their percentage was not affected much even by the injection of the strongest dose (6.0 μg β-ecdysone/Larva). Thus in case of *S. litura* the effect of β-ecdysone is much more progressive after long interval when applied insects moults to next larval instar and further in the pupal stage.

According to Ahmad (1986), when different doses of β-ecdysone were ingested by the 5th instar larvae (24 hr old) of *S. litura*, the percentage of prohaemocyte in the next stage (6th instar) following moulting increased due to less damage to these cells and more destruction of other cells. Further, intensive damage occurred to adipohaemocytes, cystocytes and spherulocytes and their percentage became negligible in prepupae. The changes were dose based. When the treated larvae reached prepupal and pupal stage, prohaemocytes were in very high percentage than that of plasmatocytes, whereas, other cells were almost absent. Similarly, when 5th instar larvae of *S. litura* were injected with different doses of Makisteron A (a phytoecdysone), following their moulting to 6th instar, the percentage of adipohaemocytes, cystocytes and spherulocytes became zero even by the weakest dose (0.5 μg/larva). Prohaemocytes were more abundant than plasmatocytes but oenocytoids still formed higher percentage due to their resistance to this chemical (Ahmad 1986). As compared to Makisterone A, the effect of the muristerone was less marked particularly with respect to prohaemocytes and plasmatocytes. However, in

the present case the hormone was topically applied and the concentrations may not be comparable to the doses used by Ahmad.

From the data of the present investigations and the observations of other investigators, it can be concluded that population of different types of haemocytes as well as the total haemocyte count/mm^3 in general following the treatment with different chemicals (insecticides and hormones) is the outcome of effects of various factors viz. (i) destruction of selective types of haemocytes (2) haemopoesis (3) mobilization of haemocytes from their sedentary positions on various tissues (4) starvation or reduced feeding (5) dehydration and consequent reduction in blood volume (6) deleterious effects on other systems of insects (7) more occurrence of mitosis in certain types of haemocytes and lastly (8) the physiological state of individual insect. Some of these factors were effective due to the direct action of insecticides on haemocytes whilst others were due to the effect of insecticides on other systems which in turn influenced the haemocyte morphology, number and proportions. The application of conventional insecticides, in general, induced more histopathological abnormalities in the haemocytes than the ecdysteroid and the juvenoid used presently. The insecticides selected in the present investigation had almost similar effect on the morphology of haemocytes of both *D. cingulatus* and *D. obliqua*. However, haemocytes of *D. cingulatus* were more susceptible to these chemicals than those of *D. obliqua*. The effect of the lower concentrations became less pronounced as time interval between application and observation increased. In the subsequent stage (after moulting) the influence of conventional insecticides (acephate, aminocarb and cypermethrin) on the haemocytes became almost negligible. This is possible on account of recovery from the toxic effect in the surviving insects and simultaneously differentiation of new haemocytes. However, in the insects treated with the ecdysteroid and the juvenoid, the changes in the haemocytes were significant in the subsequent stage also and these effects were more pronounced in the hemimetabolous insect i.e. *Dysdercus cingulatus*. Even though the variations in the total and differential counts were very high and the statistical analysis gave insignificant results. however, a definite trend of response of haemocytes was available in the present investigation which was supported by the findings of other workers as well. Conclusively, it seems appropriate to suggest that since haemocytes play very important role in defense as well as in

intermediary metabolism which are two vital mechanisms for the well being of the insects, the behavior and response of the haemocytes to different chemicals can be manipulated for use in the integrated pest management.

VI- SUMMARY

01. The objective of present investigation was to understand the comparative effects of a few insecticides representing different chemical groups on the haemocytes, if any, of *Dysdercus cingulatus* and *Diacrisia obliqua*. It was expected that such a study would reveal fundamental and additional knowledge on insect haemocytes in general and on that of aforesaid species in particular.

02. The selection of the aforesaid species (*D. cingulatus* & *D. obliqua)* was also based on the nature of their different feeding habits, growth, type of metamorphosis i.e. incomplete and complete respectively and also their importance as polyphagous pests of agricultural crops. The former species is a cosmopolitan pest of cotton and other malvaceous plants including certain vegetables, whereas, the latter species is vociferous polyphagous eater of several crops at their larval stages.

03. In the present program of research last nymphal instar in case of *D. cingulatus* and last larval instar of *D. obliqua* were chosen for re-examination of the normal haemocytes as well as haemocyte picture i.e. structure, total number (THC) and percentage of different types (DHC) following the application of the selected chemicals. The last nymphal instar of *D. cingulatus* and last larval instar of *D. obliqua* also represent full growth in the respective instars as well as their ecdysis would form a part of metamorphosis and at this stage the haemocyte picture of both the species would further throw light on their fate at metamorphosis.

04. The haemocytes of 5^{th} instar nymphs and newly emerged adults of *Dysdercus cingulatus* were classified in 5 types viz. prohaemocytes,

plasmatocytes, granulocytes, oenocytoids, and adipohaemocytes on the basis of morphology, staining and presence of cytoplasmic inclusions under light microscope following the staining of haemocytes with Giemsa's and Leishmann's stain separately.

05. The haemocytes of 6th instar larvae of *Diacrisia obliqua* were classified into 6 types viz. prohaemocytes, plasmatocytes, granulocytes, spherulocytes, oenocytoids and coagulocytes whereas in the pupae (aged 1-2 days) prohaemocytes and plasmatocytes were the predominant types and coagulocytes were occasionally present.

06. The haemocytes of the 6th instar larvae of *Diacrisia obliqua* were also studied ultrastructurally under TEM and classified into five groups: prohaemocytes, plasmatocytes, granulocytes, oenocytoids and spherulocytes. Prohaemocytes characteristically contained relatively few cytoplasmic organelles and large nucleus as compared to other cells. Plasmatocytes were polymorphic containing well-developed organelles including phagocytic vacuoles, myelinated bodies, small vacuoles and sometimes one to few granules. Granulocytes were also polymorphic but rarely spindle shaped and were characterized by the presence of few to numerous granules, well developed organelles including phagocytic vacuoles. Oenocytoids were relatively large cells with characteristically eccentric and small nucleus, dense homogeneous and fibrous cytoplasmic matrix and very few organelles. Spherulocytes were characterized by the presence of a few to many spherules in cytoplasm relatively few organelles and small vacuoles. Some intermediate cell types were also encountered.

07. The changes in total haemocyte counts (THC) were observed throughout the last nymphal instar as well as in newly emerged adult males and females of *Dysdercus cingulatus*. The THC of 1-day-old nymphs was

4557/mm^3, which progressively increased up to five days and reached to 9499.5/mm^3, and thereafter declined to 6212.3/mm^3 just before next moulting. The newly emerged females showed higher THC than males of corresponding age.

08. Similarly newly ecdysed 6th instar larvae contained 34716cells/mm^3. Subsequently the total haemocyte count increased till 4 days post ecdysis and registered 70438cells/mm^3. Thereafter the THC decreased just before ecdysis. The THC of newly formed pupae (1 to 2 day old) was significantly low (9464cells/mm3).

09. THC of last instar larvae of *Diacrisia obliqua* was significantly higher than those of last instar nymphs of *Dysdercus cingulatus* of corresponding age.

10. DHC of last nymphal instar of *Dysdercus cingulatus* determined at 1 day,2 days, 4 days and 6 days post ecdysis exhibited an increase in prohaemocytes up to four days (average population 15.44%) then dropped to 5.92% at 6th day i.e. just before moulting and in the newly emerged adult males and females it was 3.15% and 5.62% respectively. The plasmatocyte population was 50.66% in 1-day-old nymphs showing a progressive reduction till 6th day post ecdysis (36.13%). It was higher in females than in males. Adipohaemocytes population exhibited a progressive increase from 17.53% at 1 day to 35.81% at 6 days post ecdysis. Whereas in males the adipohaemocytes were significantly higher than in females. Granulocytes of 6th instar larvae showed an inconsistent change in population throughout the last larval instar. However, in adult males it was comparatively low. And finally oenocytoids showed almost constant population throughout last nymphal instar.

11. On the other hand, prohaemocyte population of 6th instar larvae of *Diacrisia obliqua* showed a progressive decline as the larvae grew older and in

the pupae it was lowest. Plasmatocytes underwent very small and inconsistent change in population throughout 6th larval instar, however, in pupae it was the highest and was represented as the predominant type. The mean granulocyte percentage rose from 2.94 in 1 day old larvae to 12.16 in 6 day old larvae. Whereas in pupae it again dropped to 3.57. Spherulocytes, oenocytoids and coagulocytes constituted only a small proportion of total haemocytes. Moreover, in pupae spherulocytes and oenocytoids were altogether absent from the smears.

12. Comparative effects of an organophosphate (Acephate), a carbamate (Aminocarb), a pyrethroid (Cypermethrin), a juvenoid (Methoprene) and an ecdysteroid (Muristerone) were investigated with regard to haemocyte morphology, THC and DHC of final nymphal instar and in the newly emerged adult of *Dysdercus cingulatus* as well as final larval instar and pupae of *Diacrisia obliqua* .

13. Only four concentrations, ranging from very low to very high (based on Lc-50 value) of each of the above-mentioned insecticides with respect to insect concerned, were topically applied for tests.

14. For *Dysdercus cingulatus* the selected concentrations of various insecticides were as follows

Acephate	:	0.006%, 0.004%, 0.002% and 0.001%
Aminocarb	:	0.008%, 0.007%, 0.004% and 0.0025%
Cypermethrin:		0.001%, 0.0008%, 0.0006% and 0.0004%
Muristerone	:	0.8%, 0.6%, 0.4% and 0.2%
Methoprene	:	0.2%, 0.1%, 0.08% and 0.04%

15. For *Diacrisia obliqua* the four selected concentrations of above mentioned insecticides were

Acephate	:	0.2%, 0.1%, 0.08% and 0.04%
Aminocarb	:	0.6%, 0.4%, 0.2% and 0.1%
Cypermethrin:		0.015%, 0.01%, 0.005% and 0.0025%
Muristerone	:	2.0%, 1.5%, 1.0% and 0.5%
Methoprene	:	1.0%, 0.8%, 0.6% and 0.4%

16. Each of the selected concentrations of above mentioned compounds was topically applied through a tuberculin syringe fitted with a manually operated micro-applicator in a uniform volume (1 µl) on individual 5^{th} instar nymphs (one day old) of *Dysdercus cingulatus* and 2 µl on 6^{th} instar larvae of *Diacrisia obliqua* on the dorsum of their prothorax.

17. The effects of each of the selected concentrations were studied 6 hrs, 1day, 3 days and 5 days post application on the haemocyte morphology, THC and DHC of *Dysdercus cingulatus* as well as *Diacrisia obliqua*. Moreover long term effects on the haemocytes of insects of the subsequent stage were also determined.

18. The effects on the haemocytes were based on the concentration of insecticide applied. The pathological symptoms were mild and were characterized by vacuolization of cytoplasm, irregular cell membrane that resulted in distortion of cell shape, occasional vacuolization of nucleus and formation of cytoplasmic extensions in relatively few haemocytes when treatment was made with lower concentrations. Whereas following higher concentrations, the pharmacological symptoms extended to a large population of haemocytes and became more severe as characterized by intense vacuolization of cytoplasm as well as nuclei, broken cell membrane at several

places, distortion in shape of cells, discharge of cell contents into the surroundings and loss of identity etc. The haemolymph became very thick and clumping of the haemocytes was also recorded.

19. The corresponding concentrations of three conventional insecticides viz Acephate, Aminocarb and Cypermethrin induced almost similar pathological symptoms in haemocytes whereas Insect Growth Regulators viz. Methoprene (analogue of juvenile hormone) and Muristerone (a phytoecdysone) did not cause such extensive damage as induced by above mentioned three insecticides.

20. The various morphological abnormalities induced in haemocytes of *Diacrisia obliqua* subsequent to the insecticidal treatment were also examined under Transmission Electron Microscope (TEM). The adverse effects were characteristically represented by vacuolization of cytoplasm (mild or intense), rupturing of cell membrane, disintegration of cytoplasmic organelles, nuclear distortion and disintegration, precipitation of chromatin material, discharge of nuclear contents into the cytoplasm and fragmentation of nucleus etc. In addition to these, many haemocytes were found to lose their identity. Thus the pathological symptoms observed under the light microscope were verified by TEM.

21. The treatment of nymphs with lower concentrations of selected insecticides generally induced slight increase in THCs whereas higher concentrations caused significant reductions in THCs at various time intervals following application of insecticides.

22. The highest concentration of acephate (0.006%) on *D. cingulatus* resulted in 43.55%, 43.56%, 67.40% and 67.54% reduction in THC at 6 hrs, 1 day, 3 days and 5 days post treatment compared to control.

23. The highest concentration of aminocarb (0.008%) caused a fall of 58.28%, 74.74%, 81.68% and 77.51% in THC at 6 hrs, 1 day, 3 days and 5 days post treatment respectively, compared to the corresponding controls.

24. The treatment with the highest concentration of cypermethrin, the reduction in THCs at 6 hrs 1 day, 3 days and 5 days post-treatment was 58.26%, 61.99%, 70.79% and 66.97% as compared to control.

25. The THCs of *Diacrisia obliqua* affected with the lowest concentrations of insecticides generally exhibited slight increase whereas the highest concentrations of the respective insecticides induced a significant reduction in cell count.

26. The highest concentration (0.02%) of acephate on *Diacrisia obliqua* larvae induced 57.07%, 77.18%, 64.33% reduction respectively at 6 hrs, 1 day, and 5 days post treatment as compared to control.

27. The highest concentration of aminocarb (0.6%) caused 64.24%, 79.06% and 68.69% fall in THC/mm^3 after 6 hrs, 1 day and 5 days respectively following the application on 6^{th} instar larvae of *D. obliqua*.

28. Similarly, the THC showed 70.82%, 77.16%, 85.71% and 81.99% reduction respectively at 6 hrs, 1 day 3 days and 5 days post-treatment with the highest concentration of cypermethrin (0.015%).

29. When the highest concentration of muristerone (0.8%) was applied on 5th instar nymphs of *Dysdercus cingulatus*, the reduction in THC was by 40.73%, 52.43%, and 57.73% respectively after 6 hrs, 1 day and 3 days post-treatment compared to control.

30. Whereas following the application of the highest concentration of methoprene (0.2%) the THC showed 31.38%, 18.99%, 36.67% and 24.86% reduction respectively after 6 hrs, 1 day, 3 days and 5 days post treatment as compared to the corresponding control.

31. The lower concentrations (0.5%, 1.0% and 1.5%) of muristerone when applied on 6th instar larvae of *D. obliqua* elicited an increase in the THCs/mm^3 of haemolymph. However, the highest concentration (2.0%) caused a reduction after an interval of 6 hrs, 1 day and 3 days post-treatment.

32. Except the lowest (0.4%), all the other selected concentrations viz., 0.6%, 0.8% and 1.0% of methoprene when applied on 6th instar larvae of *D. obliqua* resulted in the reduction of THCs respectively at 6 hrs, 1 day, 3 days and 5 days post treatment.

33. The changes in THC of *Diacrisia obliqua* following the application of methoprene and muristerone on the 6th instar larvae were not as significant as those caused by insecticides.

34. The application of various concentrations of selected insecticides viz., acephate, aminocarb and cypermethrin on 5th instar nymphs of *Dysdercus cingulatus* resulted in alterations in the proportion of various types of haemocytes. The prohaemocyte population was found to be apparently increased with increase in concentration of the insecticides applied, whereas, the percentage of plasmatocytes and adipohaemocytes showed a progressive reduction. The adipohaemocytes generally disappeared from the blood smears of insects affected with higher concentrations. The granulocytes exhibited an inconsistent change in the population with respect to increasing concentrations. The oenocytoids were most resistant cells to insecticidal treatment and did not show disintegration even at the highest concentrations. Their relative proportion apparently increased due to reduction in the percentage of other cell types.

35. The application of muristerone on 5th instar nymphs of *Dysdercus cingulatus*, exhibited a slightly different picture of DHC. The prohaemocytes showed a reduction after 6 hrs of treatment whereas adipohaemocyte percentage was enhanced at this stage. Moreover, blood picture at one day and 3 days post-treatment revealed only slight variation in prohaemocyte population. The population of plasmatocytes increased slightly and that of disintegrating cells enhanced significantly with increase in the concentration of muristeron. When the treated nymphs moulted to adult stage, the prohaemocyte population was significantly higher than before.

36. The lower concentrations (0,04%, 0.08% and 0.1%) of methoprene caused insignificant changes in the population of various types of haemocytes. Whereas, Adipohaemocytes and granulocytes showed significant reduction by the application of highest concentration i.e. 0.2%. When the nymphs moulted to adult stage the population of prohaemocytes showed significant increase by even lower concentrations.

37. Following the application with increasing concentrations of insecticides viz., acephate, aminocarb and cypermethrin on 6th instar larvae of *Diacrisia obliqua,* prohaemocytes generally exhibited a progressive rise in population, whereas, plasmatocytes showed an inverse response. With the respective higher concentrations of above-mentioned insecticides, the spherulocytes and granulocytes were mostly damaged. On the other hand, the disintegrating haemocytes were significantly enhanced. In the pupae, successfully moulted from the treated larvae, the proportion of different types of haemocytes exhibited inconsistent changes as compared to control. Changes in population of prohaemocytes, granulocytes and disintegrating cells were sometimes significant in the pupae affected with higher concentrations.

38. The application of different concentrations of muristerone and methoprene caused rise in the population of prohaemocytes and fall in that of plasmatocytes. Unlike above-mentioned insecticides, the exogenous hormone treatment generally induced an increase in the population of granulocytes. Although the respective highest concentrations of muristerone and methoprene significantly increased the proportion of disintegrating haemocytes, it was nevertheless lower than that of insecticide treated larvae. Oenocytoids and coagulocytes generally registered slight and insignificant increase.

LIST OF ABBREVIATIONS

I. Numbers Abbreviated in Plates

Coagulated haemolymph	1
Coalescence of tentacles	2
Discharge of nuclear contents into cytoplasm	3
Disintegrated chromatin material	4
Disrupted nuclear membrane	5
Excessively disrupted cytoplasm	6
Extensive vacuolization	7
Fragmented nucleus	8
Frayed and torn cytoplasm	9
Frayed and torn nucleus	10
Indistinct cytoplasm and nucleus	11
Indistinct cytoplasmic organelles	12
Indistinct heterochromatin and euchromatin	13
Irregular cell boundaries	14
Lysed organelles	15
Pseudopod	16
Ragged appearance	17
Tentacles	18
Shrinked nucleus	19
Swollen nucleus	20
Cytoplasmic extension	21
Cell clumping	22

II. Abbreviations for Plates

PR	Prohaemocytes
PL	Plasmatocytes
AD	Adipohaemocytes
GR	Granulocytes
OE	Oenocytoids
CO	Coagulocytes
SP	Spherulocytes
V	Vacuole
N	Nucleus
Cy	Cytoplasm
Gr	Granules
gl	Globules
chr	Chromatin material
chrs	Chromosomes
EPr	Early Prophase
LPr	Late Prophase
MP	Metaphase
AP	Anaphase
TP	Telophase
LTP	Late Telophase
Cyt	Cytokinesis
DC	Damaged Cell

PLATE-1

Haemocytes of 5th instar nymph of normal *Dysdercus cingulatus* under light microscope.

Fig A. Different types of haemocytes

Fig B. Normal haemocytes at higher magnification.

Fig C. Showing prohaemocytes and granulocytes.

Fig D. Showing prohaemocyte, plasmatocyte and oenocytoid.

Fig E. Showing plasmatocytes.

Fig F. Many round and spindle shaped plasmatocytes and prohaemocytes.

Fig G. Numerous prohaemocytes and plasmatocytes.

Fig H. Showing oenocytoids and plasmatocytes.

Fig I. Plasmatocytes and granulocytes with numerous granules in cytoplasm.

PLATE-I

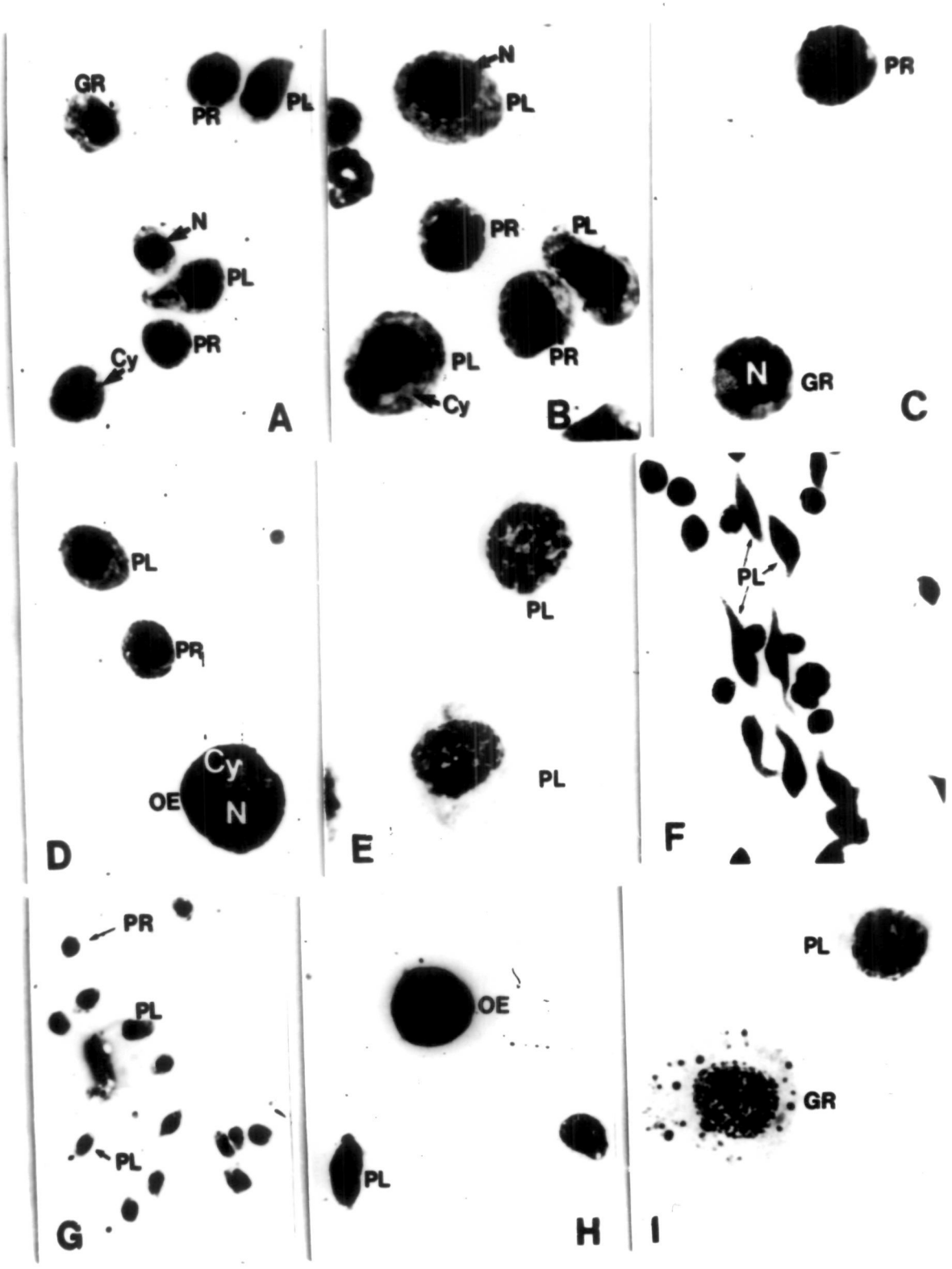
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PLATE-II

Haemocytes of 5th instar nymph of normal *Dysdercus cingulatus* under light microscope.

Fig A.	Showing granulocytes with large cytoplasmic granules and a prohaemocyte.
Fig B.	Showing adipohaemocytes in different stages of maturation.
Fig C & D.	Adipohaemocytes in different stages of maturation.
Fig E.	Mature adipohaemocyte. One adipohaemocyte completely filled with globules and partly eclipsed nucleus.
Fig F, G, H & I	Adipohaemocytes at higher magnification showing various stages of maturation.

PLATE-II

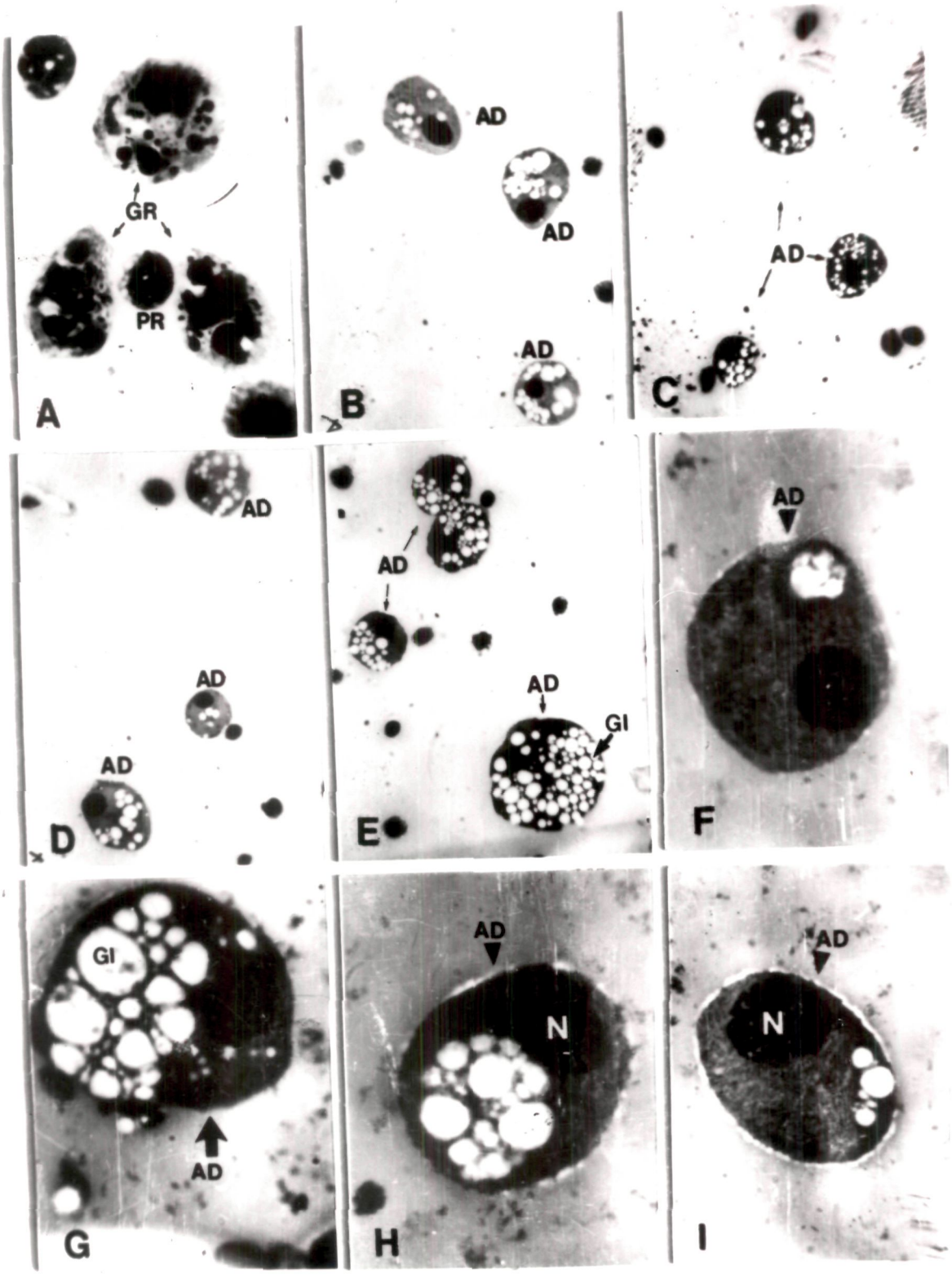

PLATE-III

Haemocytes of 5th instar nymphs and adults of normal *Dysdercus cingulatus* under light microscope.

Fig A & D.	Different types of haemocytes of adult males.
Fig B.	Showing numerous oenocytoids
Fig C.	Prohaemocytes and plasmatocytes of adult females.
Fig E to N.	Showing various stages of mitotic cell division: E. Prophase, F. Early prophase, G, H and I. Late prophase, J. Metaphase, K. Anaphase, L.Telophase, M. Late telophase, N. Cytokinesis.
Fig O.	Blood smear showing numerous flagellate like organisms.
Fig P.	Flagellate like organisms at higher magnification.
Fig Q.	Blood smear showing flagellate like organism as well as adipohaemocytes and plasmatocytes.

PLATE-III

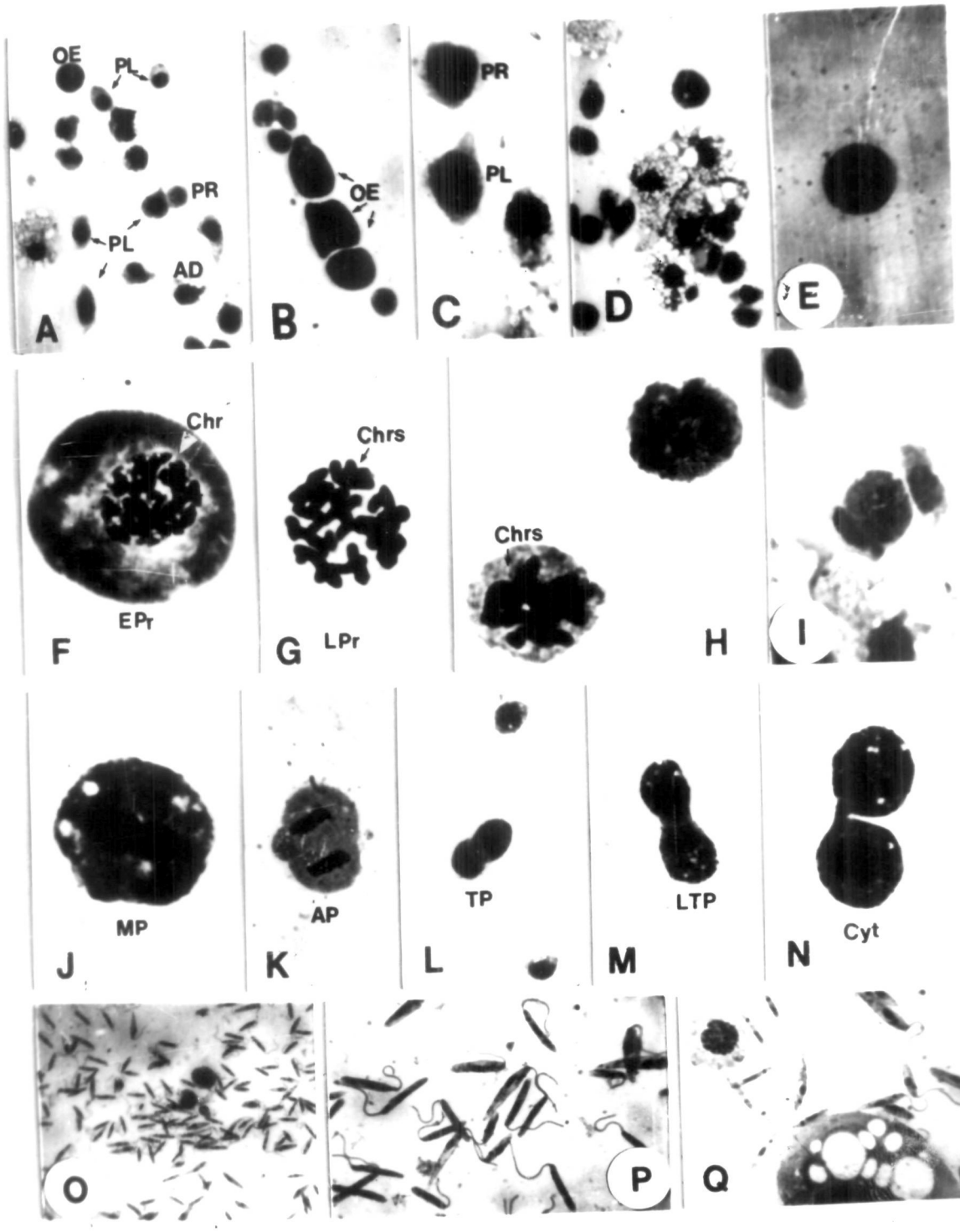
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PLATE-IV

Haemocytes of 5th instar nymphs and adults of *Dysdercus cingulatus* affected with various concentrations of acephate.

Fig A.	Haemocyte picture after 6 hrs following treatment with 0.002% acephate.
Fig. H.	Haemocyte picture after 6 hrs following treatment with 0.006% acephate.
Fig C.	Haemocyte picture after one day following treatment with 0.002% acephate.
Fig B & J.	Haemocyte picture after one day following treatment with 0.006% acephate.
Fig. L.	Haemocyte picture after 3 days following treatment with 0.004% acephate.
Fig I, K & P.	Haemocyte picture after five days following treatment with 0.004% concentration.
Fig. D,E,F,G, M,N&O.	Haemocyte picture after five days following treatment with 0.006% Acephate.

PLATE-IV

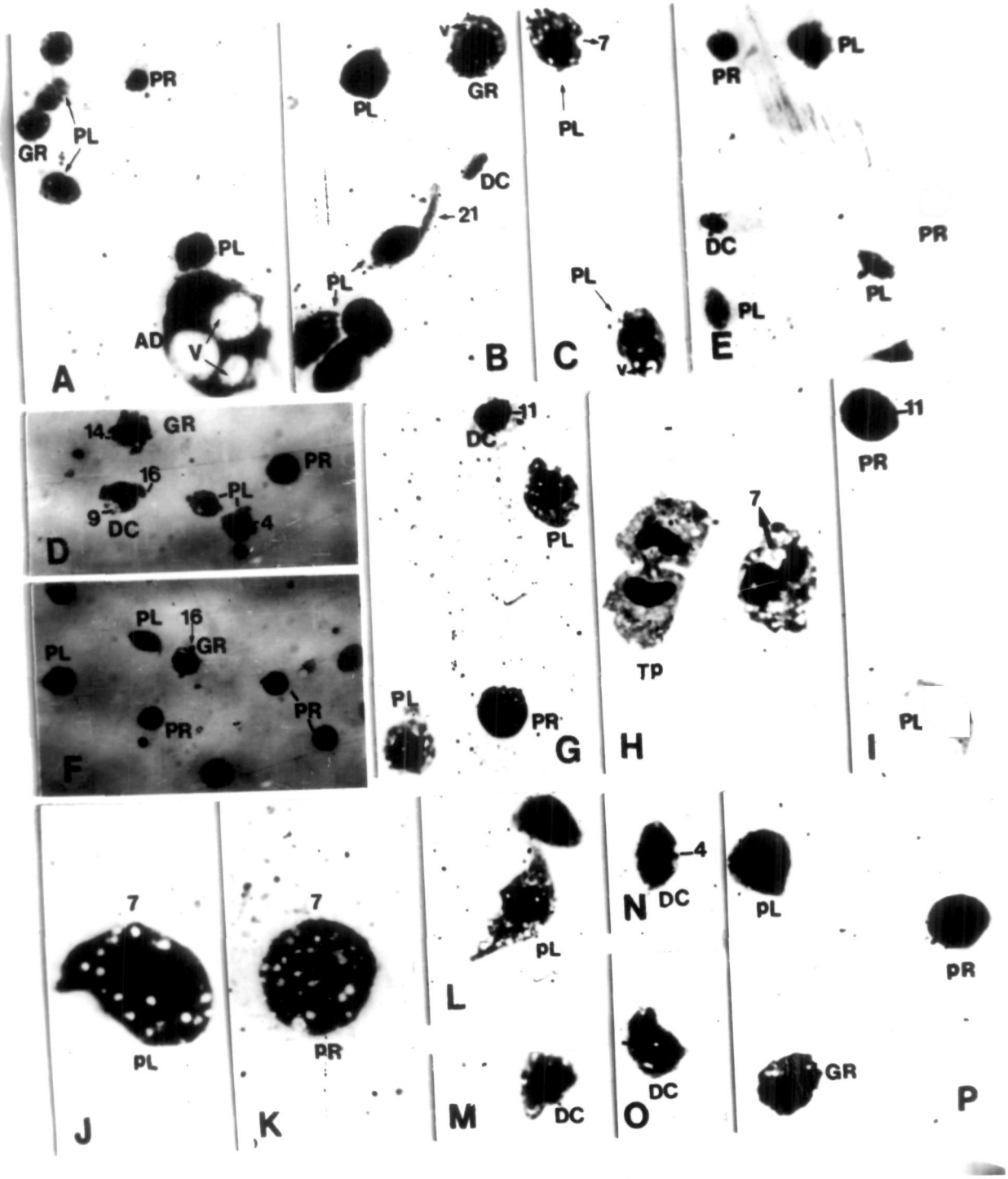
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PLATE-V

Haemocytes of 5th instar nymphs and adults of *Dysdercus cingulatus* affected with various concentrations of aminocarb

Fig. A,D&G. Haemocyte picture after 6hrs following treatment with 0.007% aminocarb.

Fig. B. Haemocyte picture after 6hrs following treatment with 0.004% aminocarb

Fig. C. Haemocyte picture after one day following treatment with 0.004% aminocarb

Fig. E. Haemocyte picture after 3 day following treatment with 0.004% aminocarb

Fig. F. Haemocyte picture after one day following treatment with 0.0025% aminocarb

Fig. H. Haemocyte picture after 3 day following treatment with 0.007% aminocarb

Fig. I. Haemocyte picture after 5 day following treatment with 0.007% aminocarb

Fig. J, K & L. Haemocyte picture after 5 day following treatment with 0.001% aminocarb

PLATE-V

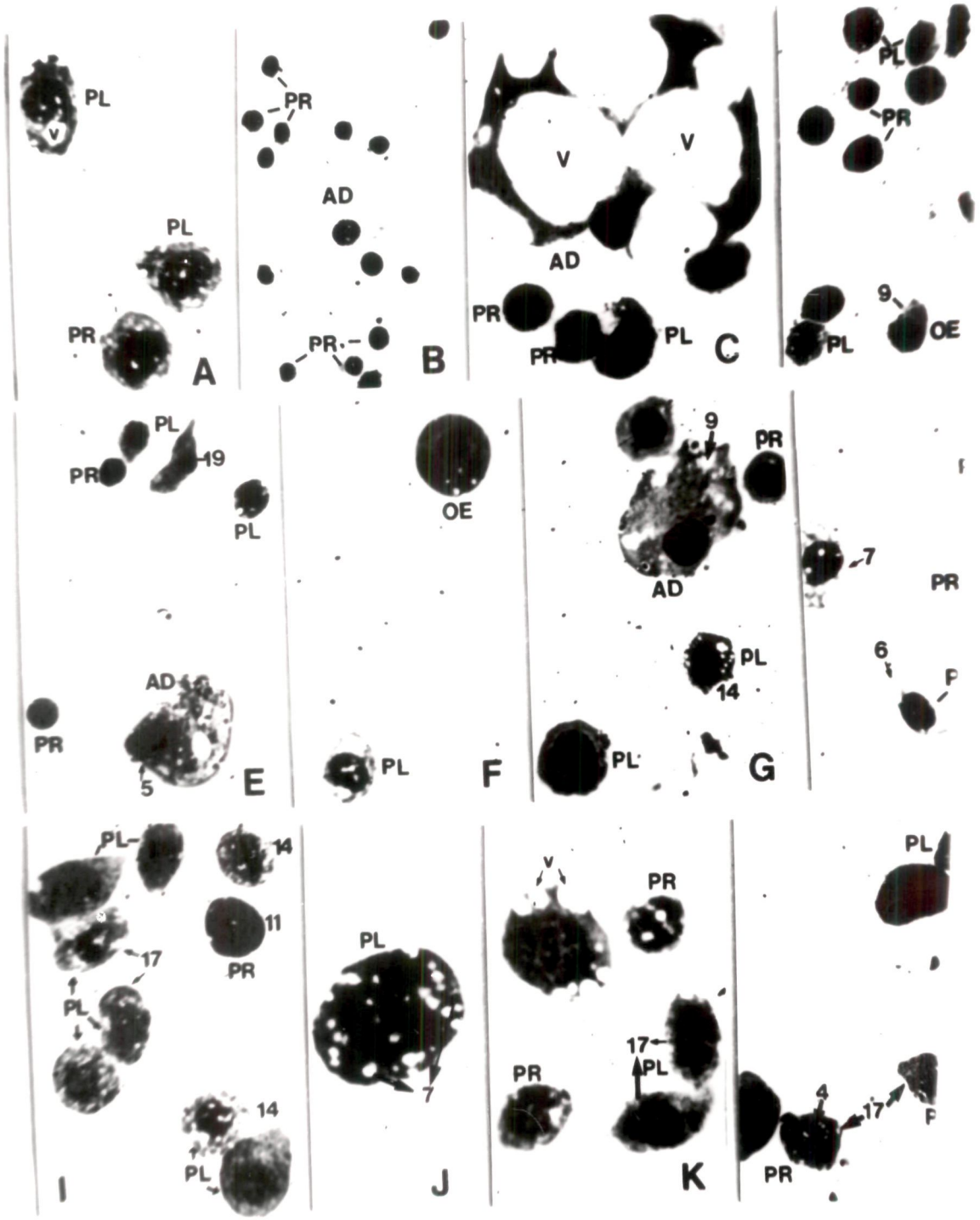

PLATE-VI

Haemocytes of 5th instar nymphs and adults of *Dysdercus cingulatus* affected with various concentrations of cypermethrin

Fig. A.	Haemocyte picture after 6 hrs following treatment with 0.0004% cypermethrin
Fig. B & D.	Haemocyte picture after 6 hrs following treatment with 0.0008% cypermethrin
Fig. C.	Haemocyte picture after one day following treatment with 0.001% cypermethrin
Fig. E.	Haemocyte picture after 3 day following treatment with 0.0006% cypermethrin
Fig. F, G & H.	Haemocyte picture after 3 day following treatment with 0.001% cypermethrin
Fig. I.	Haemocyte picture after 5 day following treatment with 0.0008% cypermethrin
Fig. J.	Haemocyte picture after 5 day following treatment with 0.0006% cypermethrin
Fig. K,L, M. & N	Haemocyte picture after 5 day following treatment with 0.001% cypermethrin

PLATE-VI

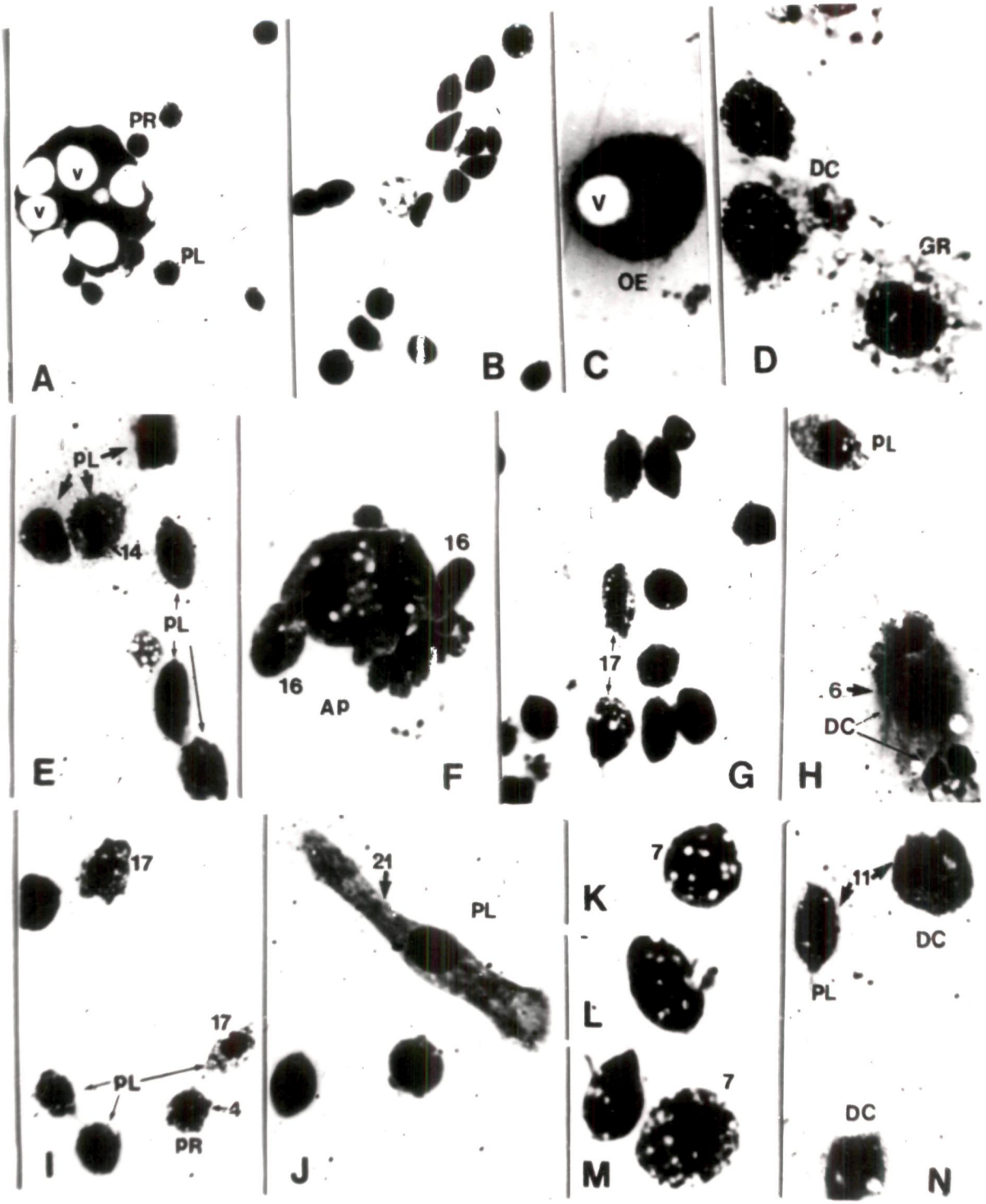
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PLATE-VII

Haemocytes of 5th instar nymphs and adults of *Dysdercus cingulatus* affected with various concentrations of muristerone

Fig. A & B.	Haemocyte picture after 6 hrs following treatment with 0.6% muristerone
Fig. C & E.	Haemocyte picture after 3 day following treatment with 0.8% muristerone
Fig. D.	Haemocyte picture after 3 day following treatment with 0.4% muristerone
Fig. F.	Haemocyte picture after one day following treatment with 0.6% muristerone
Fig. G & H.	Haemocyte picture after one day following treatment with 0.4% muristerone
Fig. I.	Haemocyte picture after 5 day following treatment with 0.4% muristerone
Fig. J K & L.	Haemocyte picture of nymphal adult intermediate following treatment with 0.8% muristerone
Fig. M.	Haemocyte picture of nymphal-adult intermediates following treatment with 0.8% muristerone

PLATE-VII

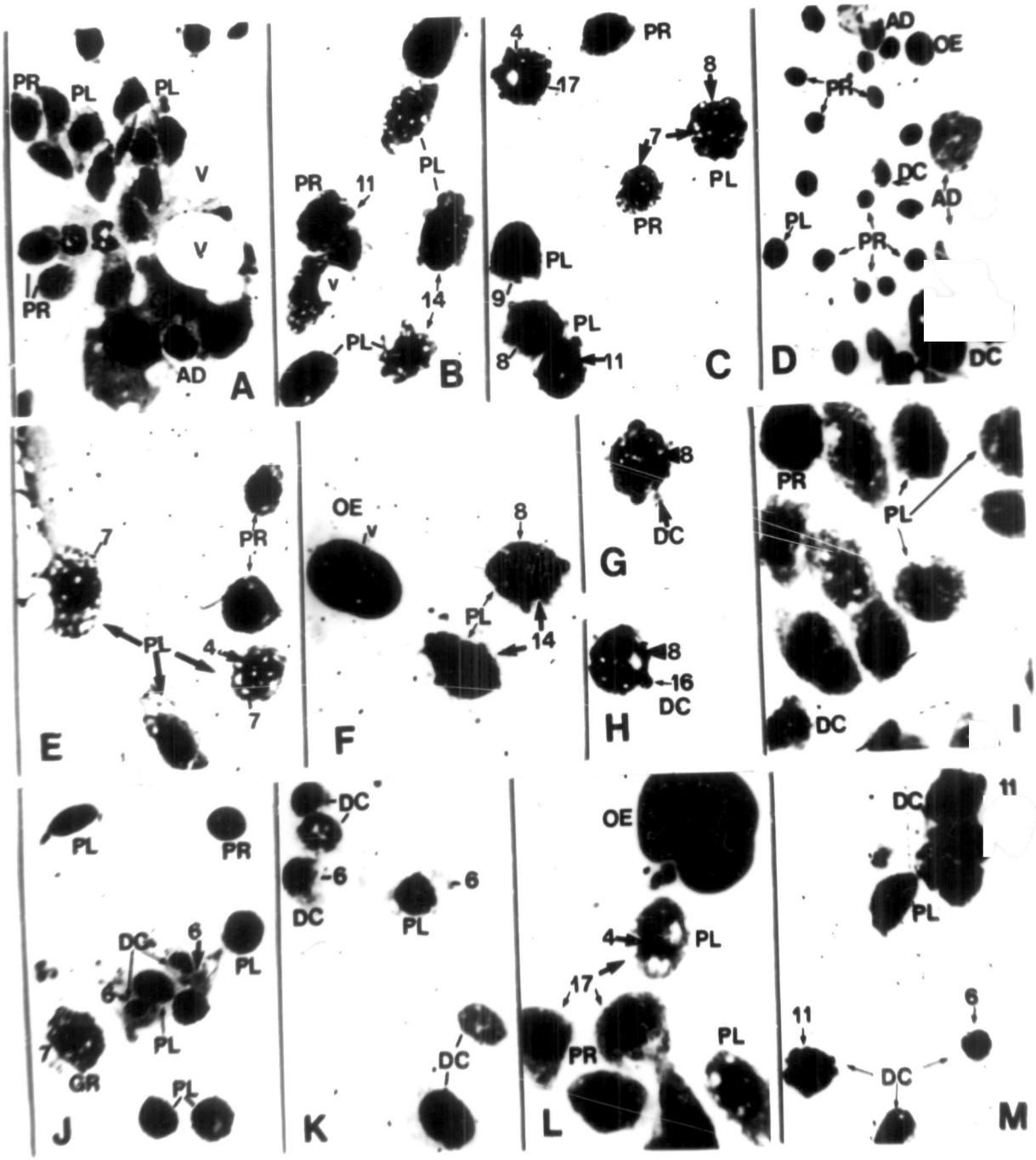
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PLATE-VIII

Haemocytes of 5th instar nymphs and adults of *Dysdercus cingulatus* affected with various concentrations of methoprene.

Fig. A, C & H.	Haemocyte picture after 5 day following treatment with 0.2% methoprene
Fig. B, G, I & K.	Haemocyte picture after one day following treatment with 0.1% methoprene
Fig. D & E.	Haemocyte picture after 6 hrs following treatment with 0.2% methoprene
Fig. F & J	Haemocyte picture after 3 day following treatment with 0.2 % methoprene
Fig. M.	Haemocyte picture of adult female affected with 0.08% methoprene
Fig. N.	Haemocyte picture of nymphal-adult intermediates following treatment with 0.2% methoprene

PLATE-VIII

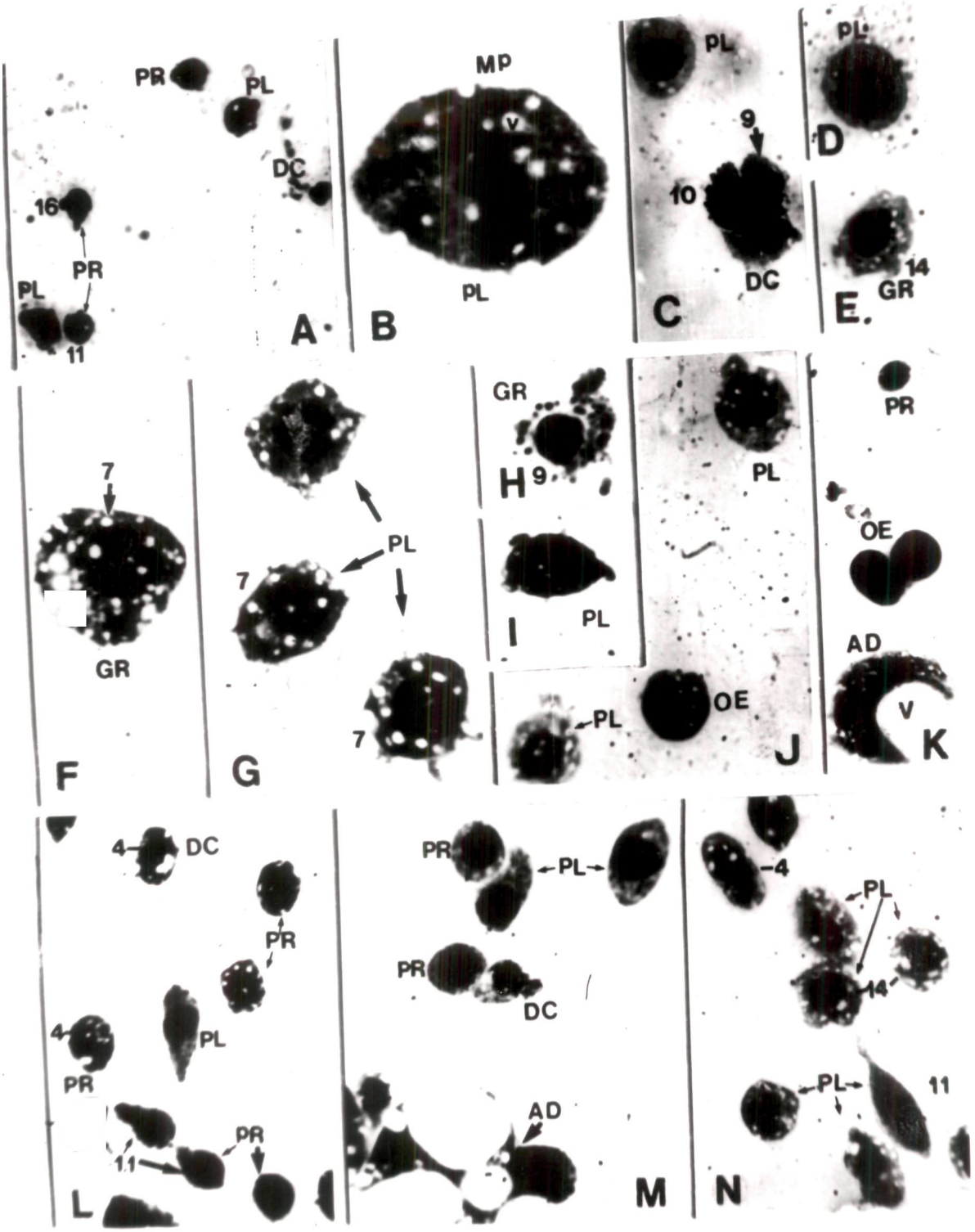
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PLATE-IX

Haemocytes of 6th instar larvae and pupae of normal *Diacrisia obliqua* under light microscope

Fig. A.	Showing spherulocytes and prohaemocytes
Fig. B.	Showing young and mature prohaemocytes
Fig. C.	Showing prohaemocytes, plasmatocyte and anaphase stage of cell division
Fig. D & E.	Showing prohaemocyte, plasmatocyte and coagulocytes
Fig. F.	Showing plasmatocytes and a haemocyte spread on slide surface
Fig. G.	Showing prohaemocyte, plasmatocyte and late anaphase/telophase stage of mitotic cell division.
Fig. H.	Showing numerous oenocytoid and coagulocytes
Fig. I.	Showing prohaemocyte and plasmatocyte as well as metaphase and anaphase stage of cell division.

PLATE-IX

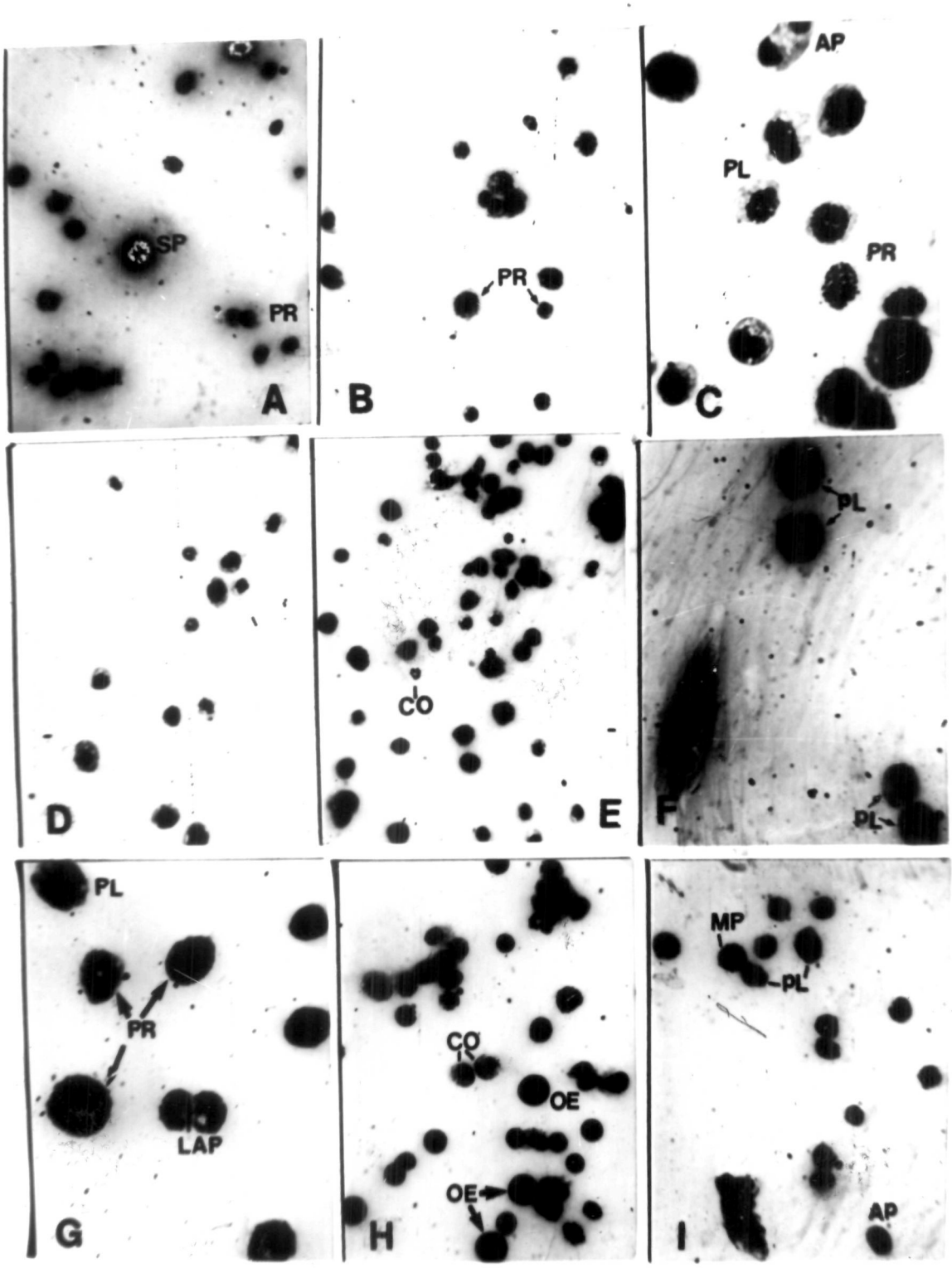

PLATE-X

Haemocytes of 6th instar larvae and pupae of *Diacrisia obliqua* affected with various concentrations of acephate

Fig. A. Haemocyte picture after 6 hrs following treatment with 0.1% acephate

Fig. B. Haemocyte picture after one day following treatment with 0.2% acephate

Fig. C & D. Haemocyte picture after 3 day following treatment with 0.1% acephate

Fig. E. Haemocyte picture after one day following treatment with 0.04% acephate

Fig. F. Haemocyte picture of pupae following treatment with 0.08% acephate

Fig. G & H. Haemocyte picture after 5 day following treatment with 0.2% acephate

PLATE-X

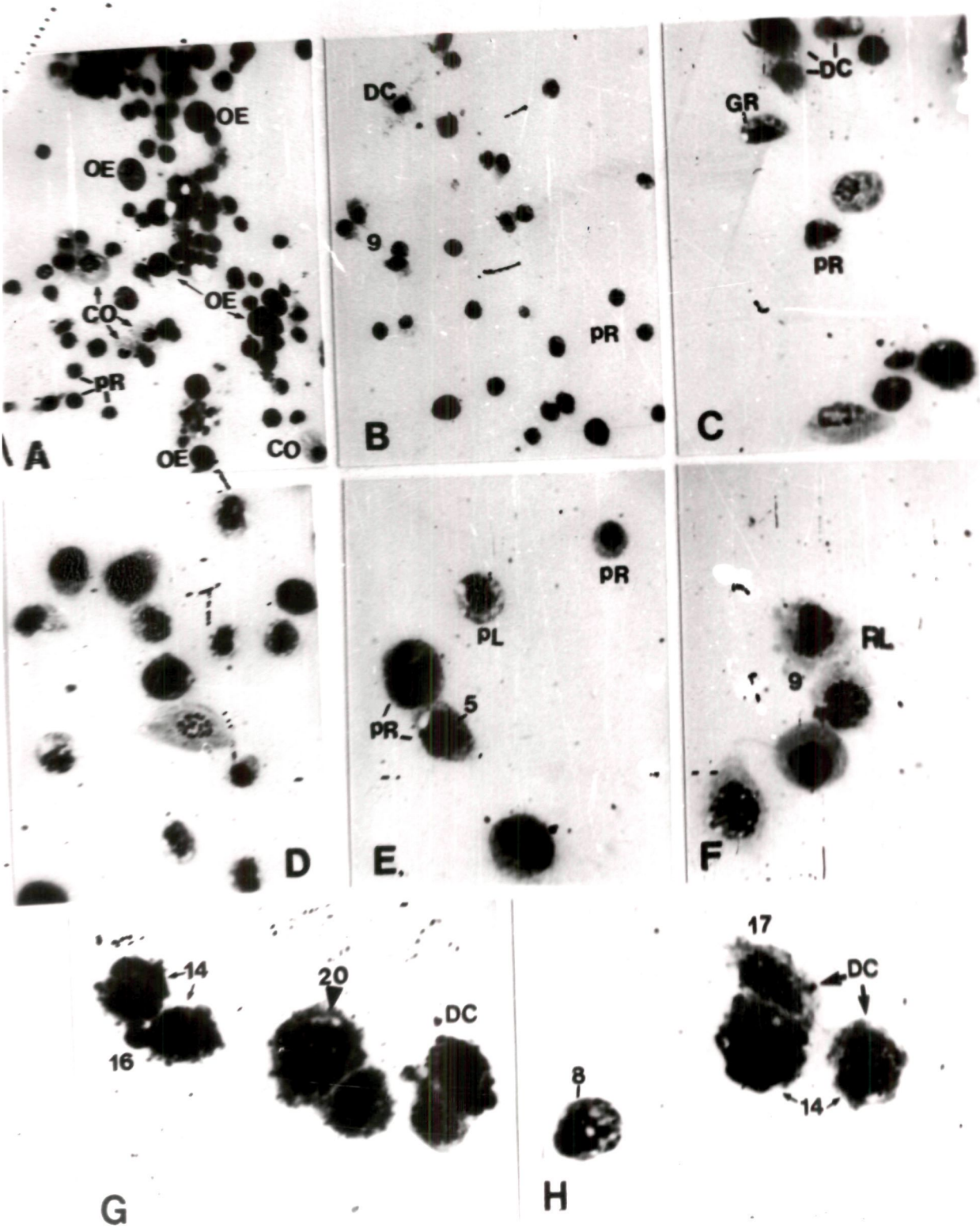
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PLATE XI

Haemocytes of 6th instar larvae and pupae of *Diacrisia obliqua* affected with various concentrations of aminocarb.

Fig. A.	Haemocyte picture after 3 day following treatment with 0.4% aminocarb
Fig. B.	Haemocyte picture after 6 hrs following treatment with 0.4% aminocarb
Fig. C.	Haemocyte picture after 6 hrs following treatment with 0.6% aminocarb
Fig. D.	Haemocyte picture after one day following treatment with 0.4% aminocarb
Fig. E.	Haemocyte picture after 3 day following treatment with 0.1% aminocarb
Fig. F & H.	Haemocyte picture after one day following treatment with 0.6% aminocarb
Fig. G.	Haemocyte picture after one day following treatment with 0.1% aminocarb
Fig. I.	Haemocyte picture after 5 day following treatment with 0.6% aminocarb

PLATE-XI

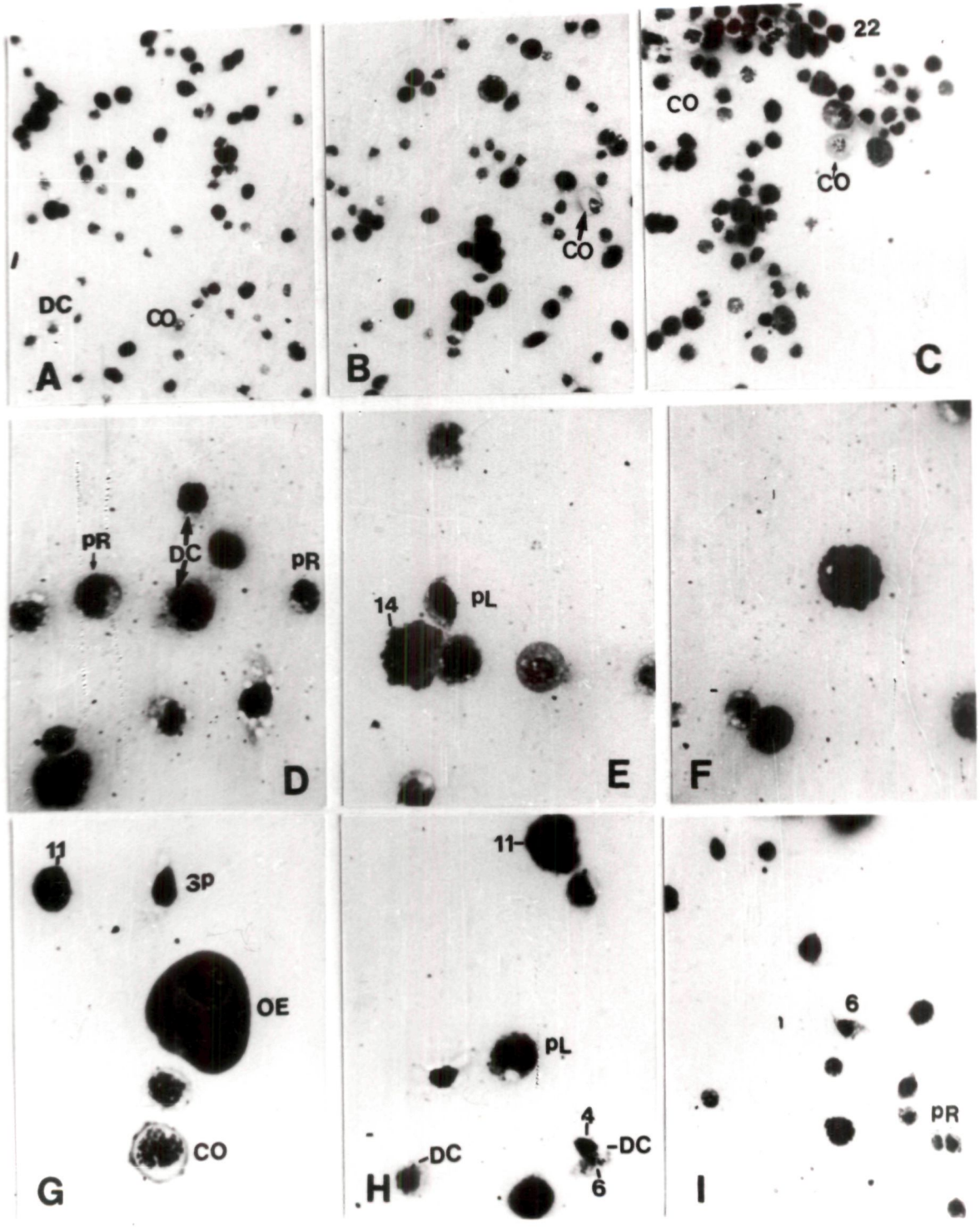
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PLATE-XII

Haemocytes of 6th instar larvae and pupae of *Diacrisia obliqua* affected with various concentrations of cypermethrin

Fig. A & B. Haemocyte picture after 6 hrs following treatment with 0.01% cypermethrin

Fig. C. Haemocyte picture after one day following treatment with 0.005% cypermethrin

Fig. D. Haemocyte picture after one day following treatment with 0.015% cypermethrin

Fig. E & F. Haemocyte picture after 3 day following treatment with 0.005% cypermethrin

Fig. G. Haemocyte picture of pupae affected with 0.0025% cypermethrin

Fig. H. Haemocyte picture after 5 day following treatment with 0.01% cypermethrin

Fig. I. Haemocyte picture after 5 day following treatment with 0.015% cypermethrin

PLATE-XII

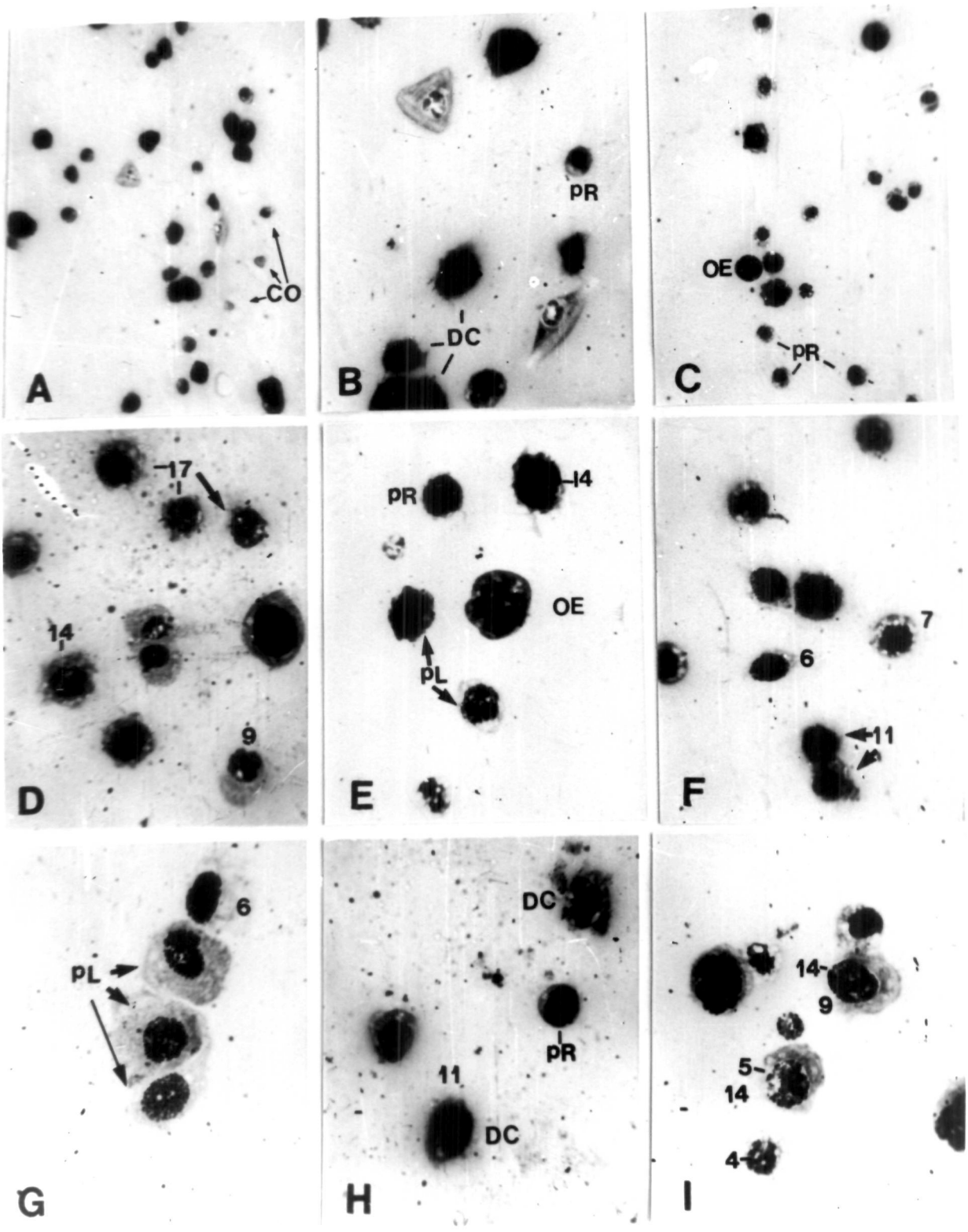
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PLATE-XIII

Haemocytes of 6th instar larvae and pupae of *Diacrisia obliqua* affected with various concentrations of muristerone

Fig. A. Haemocyte picture after one day following treatment with 1.5% muristerone

Fig. B. Haemocyte picture after 6 hrs following treatment with 2.0% muristerone

Fig. C. Haemocyte picture after 6 hrs following treatment with 2.0% muristerone

Fig. E. Haemocyte picture after 5 day following treatment with 1.5% muristerone

Fig. F. Haemocyte picture after 3 day following treatment with 2.0% muristerone

Fig. G & H. Haemocyte picture of pupae affected with 1.0% muristerone

PLATE-XIII

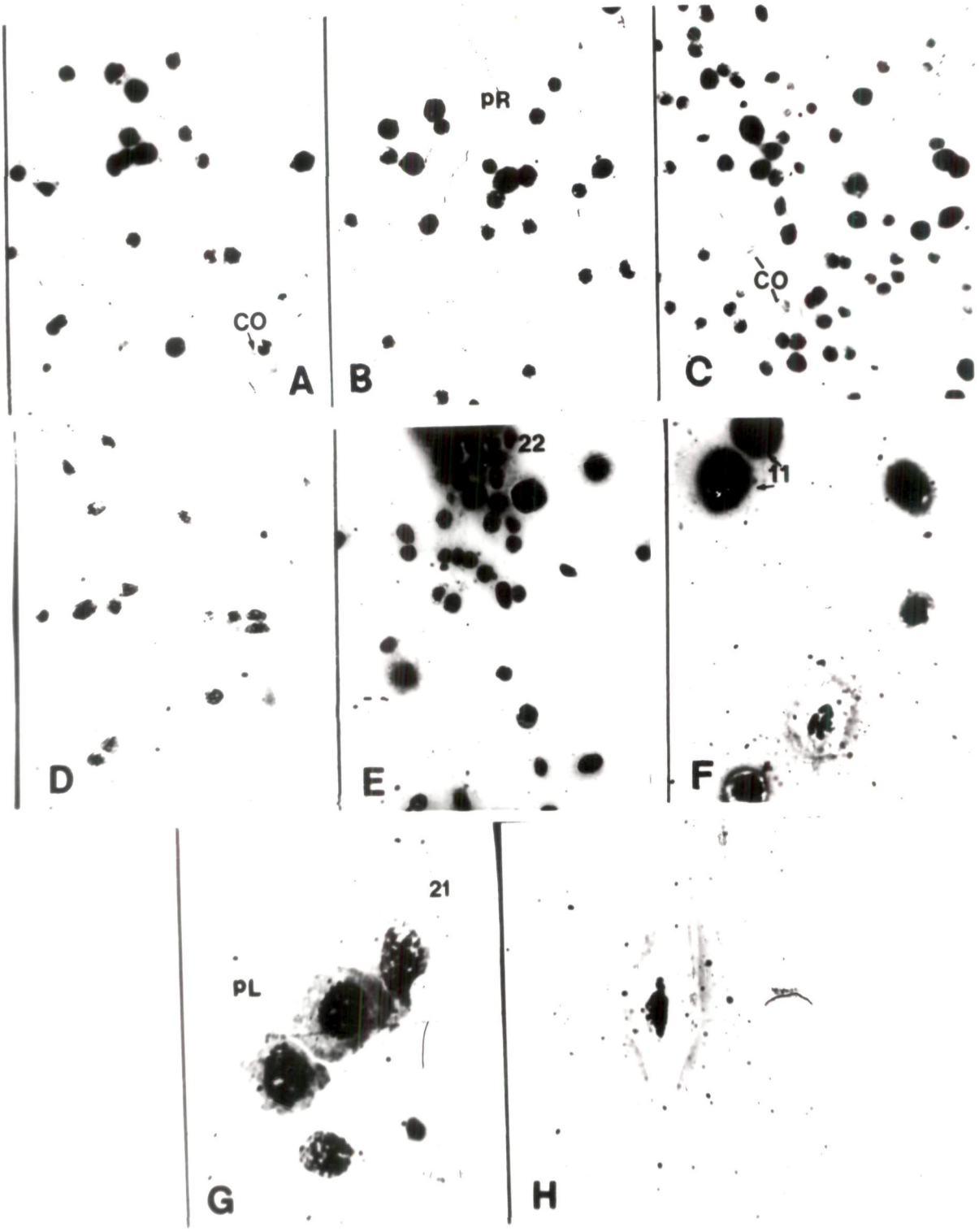


PLATE-XIV

Haemocytes of 6th instar larvae and pupae of *Diacrisia obliqua* affected with various concentrations of methoprene

Fig. A. Haemocyte picture after 6 hrs following treatment with 0.6% methoprene

Fig. B. Haemocyte picture after one day following treatment with 0.8% methoprene

Fig. C. Haemocyte picture after one day following treatment with 1.0% methoprene

Fig. D. Haemocyte picture after 3 day following treatment with 0.6% methoprene

Fig. E & F. Haemocyte picture after 5 day following treatment with 0.8% methoprene

Fig. G. Haemocyte picture of pupae affected with 0.6% methoprene

PLATE-XIV

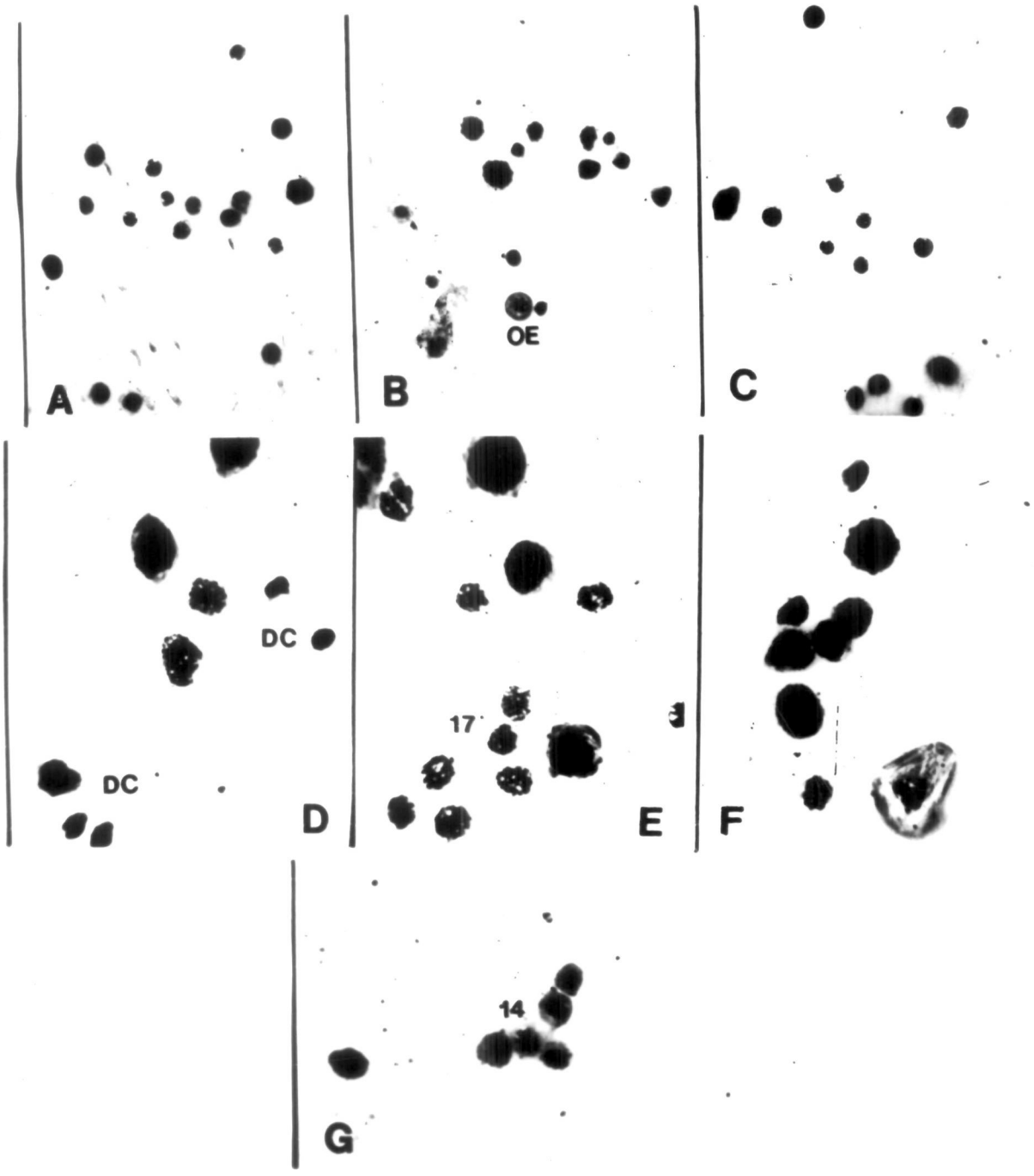


PLATE-XV

Ultrastructure of normal haemocytes under Transmission Electron Microscope (TEM), of 6^{th} instar larvae of *Diacrisia obliqua*.

Fig. A. Round prohaemocyte with large nucleus , rough endoplasmic reticulum and mitochondria.

Fig. B. Oval prohaemocyte with large round nucleus and prominent nucleolus.

Fig. C. Pear shaped prohaemocyte with nucleus, distinct nucleolus and undifferentiated cytoplasm.

Fig. D. Irregular prohaemocyte with triangular nucleus, small nucleolus and very small vacuoles in cytoplasm.

PLATE-XV

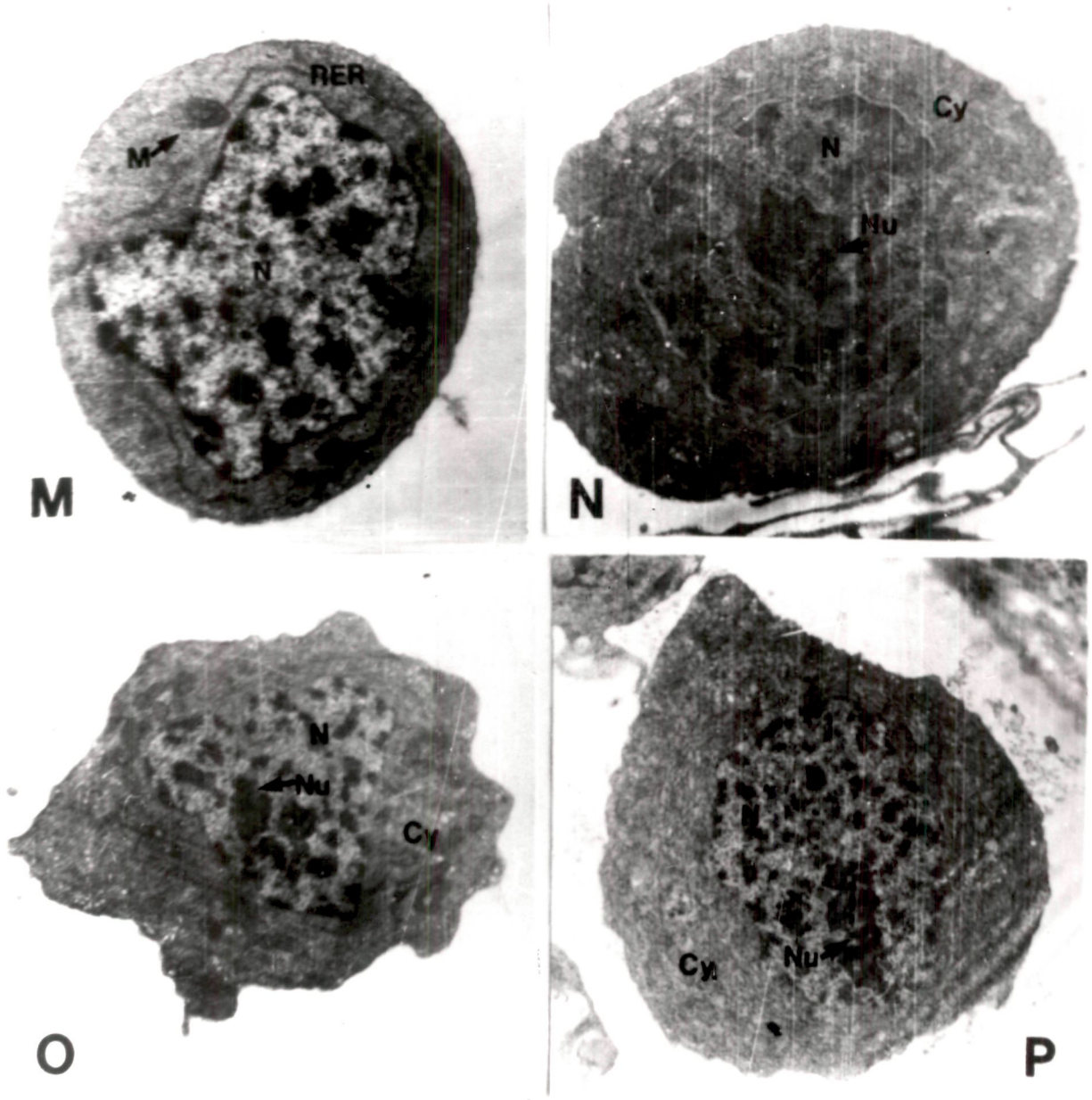


PLATE-XVI

Ultrastructure of normal haemocytes under Transmission Electron Microscope (TEM), of 6^{th} instar larvae of *Diacrisia obliqua*

Fig. A. Oval shaped plasmatocyte showing numerous mitochondria, rough endoplasmic reticulum and lysosome.

Fig. B & C. Showing spindle shaped plasmatocytes with elongated nucleus and vacuoles.

Fig. D. Oval plasmatocyte showing Golgi complex, mitochondria and a lobed nucleus.

Fig. E. Showing oval plasmatocyte and a prohaemocyte with an oval nucleus.

PLATE-XVI

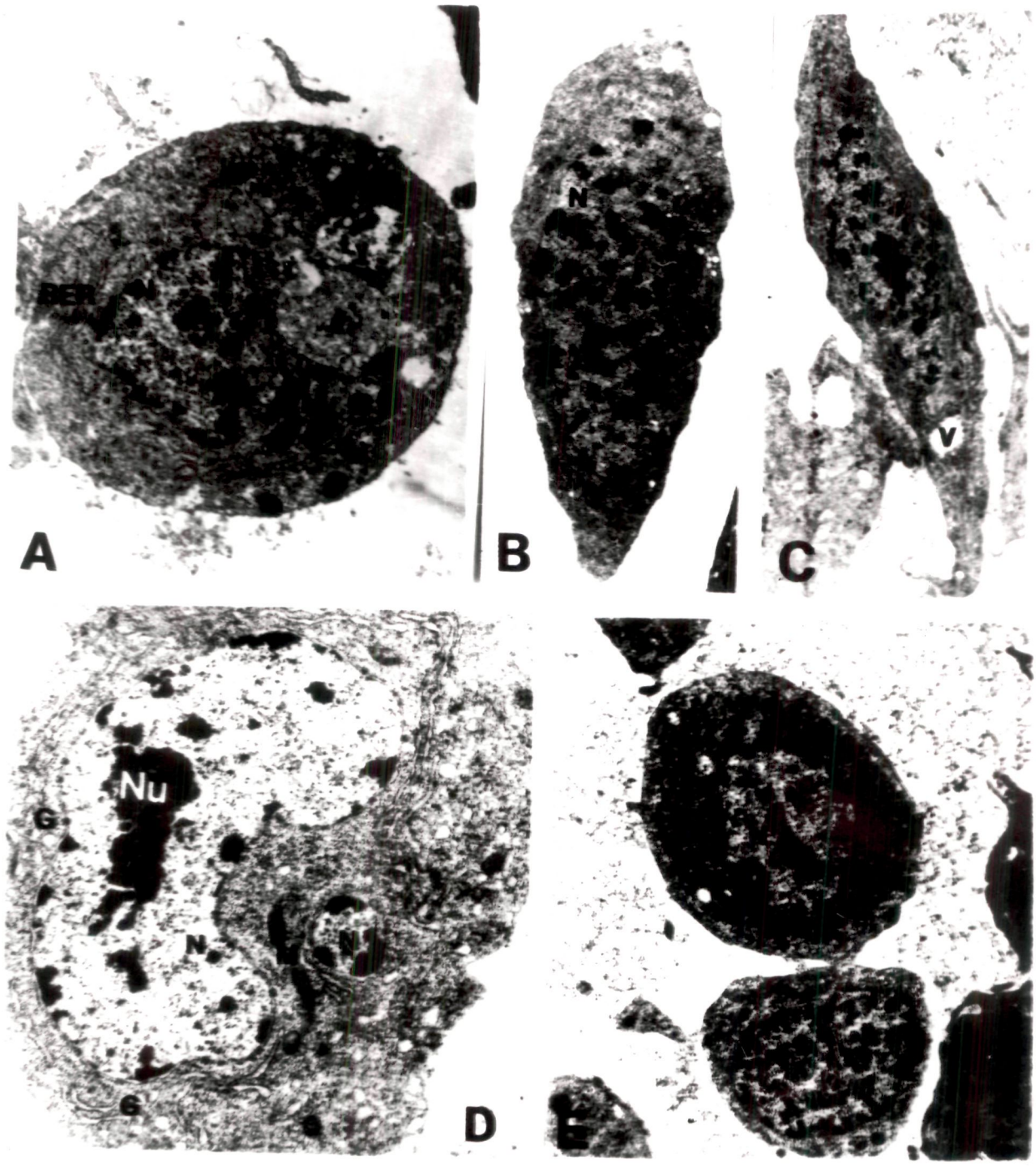


PLATE-XVII

Ultrastructure of normal haemocytes under Transmission Electron Microscope (TEM), of 6^{th} instar larvae of *Diacrisia obliqua*

Fig. A. Spindle shaped plasmatocyte showing vacuoles, mitochondria, rough endoplasmic reticulum and numerous free ribosomes in cytoplasmic metrix.

Fig. B. Plasmatocyte with membrane bound lysosome containing residual bodies (Double arrow) and rough endoplasmic reticulum having distended cisternae filled with material (Single arrow).

Fig. C. Spindle shaped plasmatocyte with numerous mitochondria, rough endoplasmic reticulum and free ribosomes.

Fig. D. Oval plasmatocyte with large nucleus having distinct heterochromatin and euchromation and a few vacuoles in the cytoplasm.

Fig. E. Spindle shaped plasmatocyte having spindle shaped nucleus with distinct heterochromatin and euchromatin and indistinct cytoplasmic organelles.

Fig. F. Magnified view of fig. C showing cytoplasm plasmatocyte filled with numerous ribosomes.

Fig. G. Elongated plasmatocyte with numerous well developed mitochondria and indistinct nucleus.

PLATE-XVII

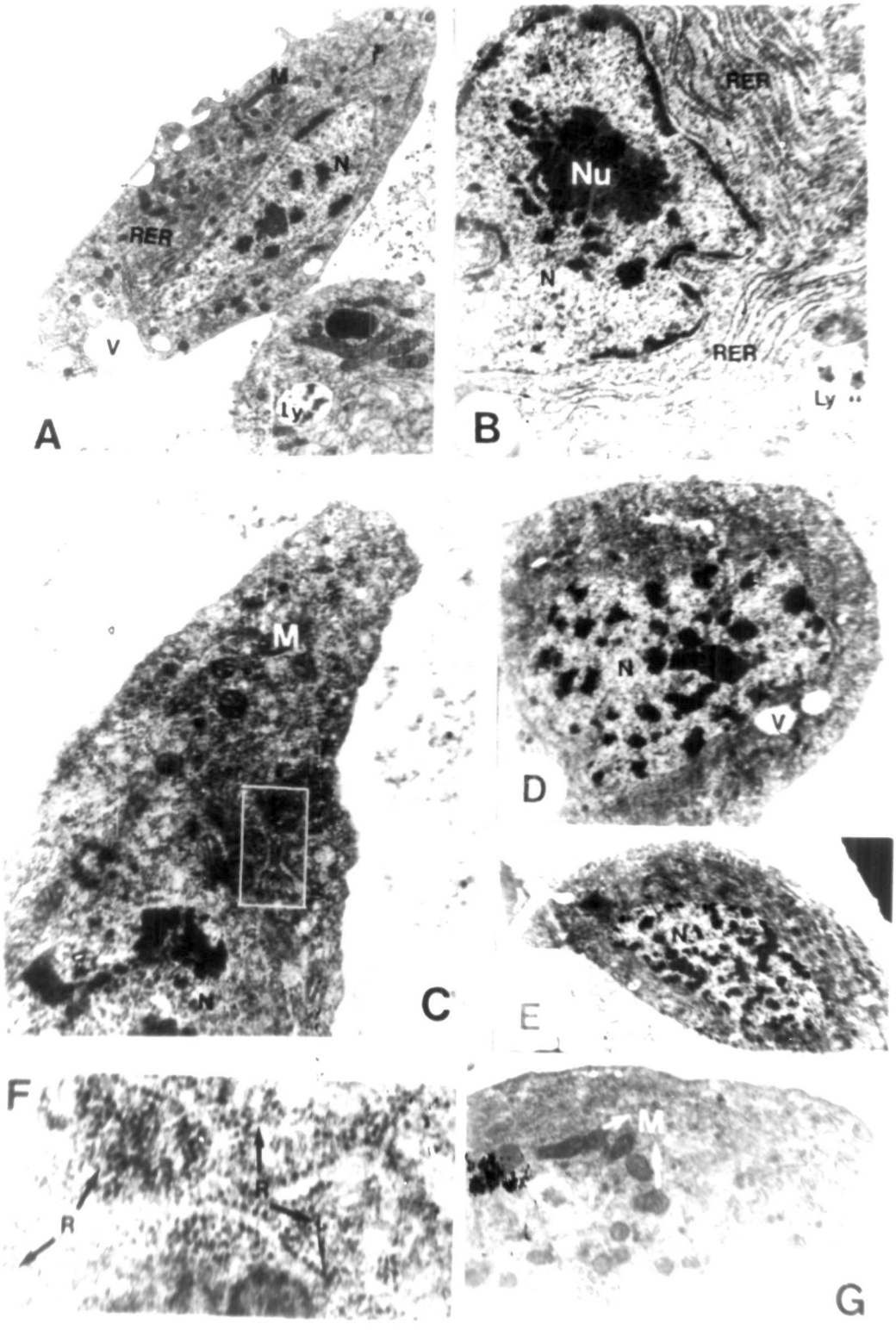


PLATE- XVIII

Ultrastructure of normal haemocytes under Transmission Electron Microscope (TEM), of 6th instar larvae of *Diacrisia obliqua.*

Fig.A. Spindle shaped plasmatocyte showing elongated nucleus with euchromatin and heterochromatin.

Fig. B. Granular haemocyte showing different types of granules, lysosomes and nucleus.

Fig. C. Showing granules of different sizes and vacuole.

Fig. D. Showing granules and elongated nucleus.

PLATE-XVIII

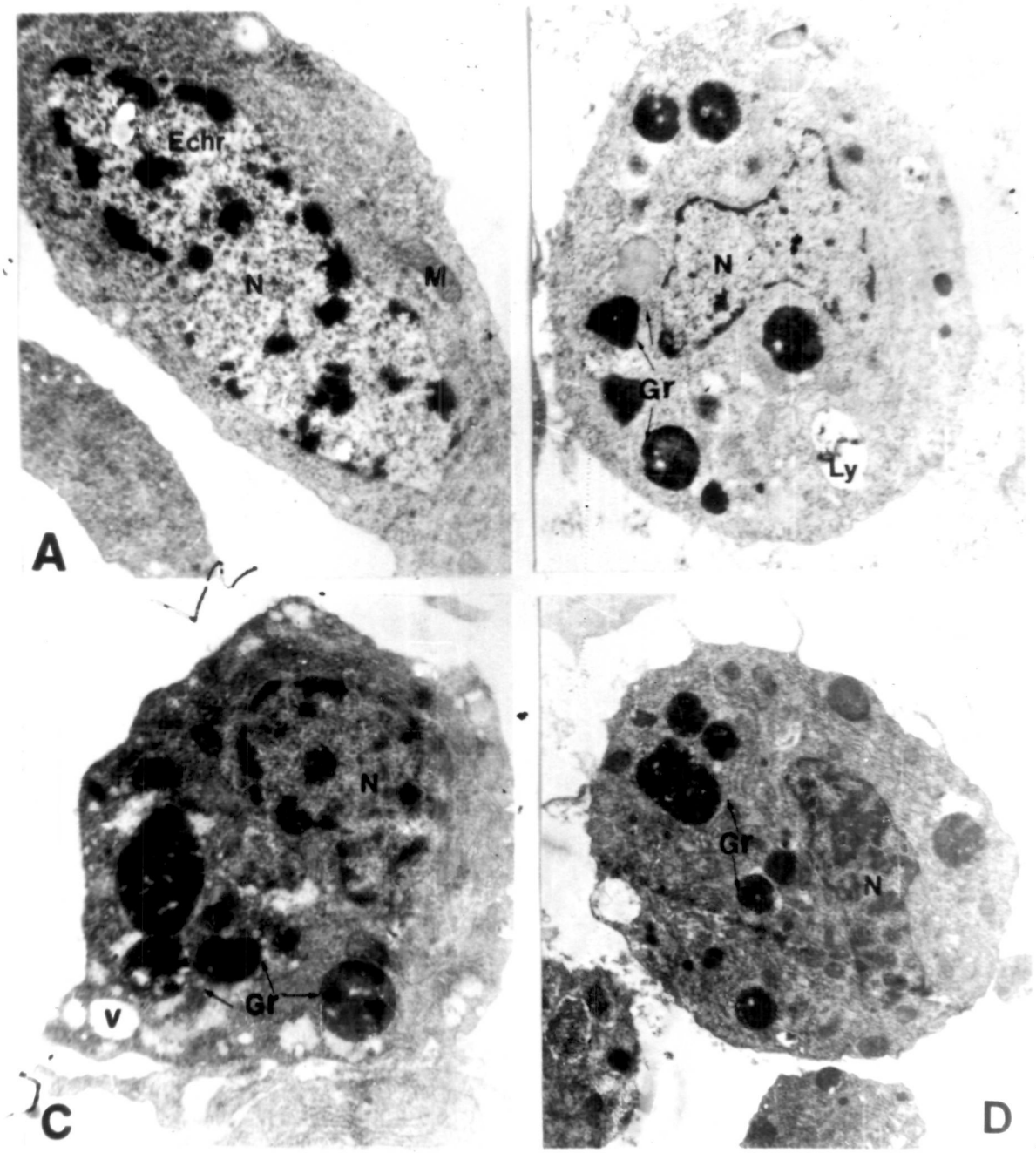


PLATE-XIX

Ultrastructure of normal haemocytes under Transmission Electron Microscope (TEM), of 6^{th} instar larvae of *Diacrisia obliqua*.

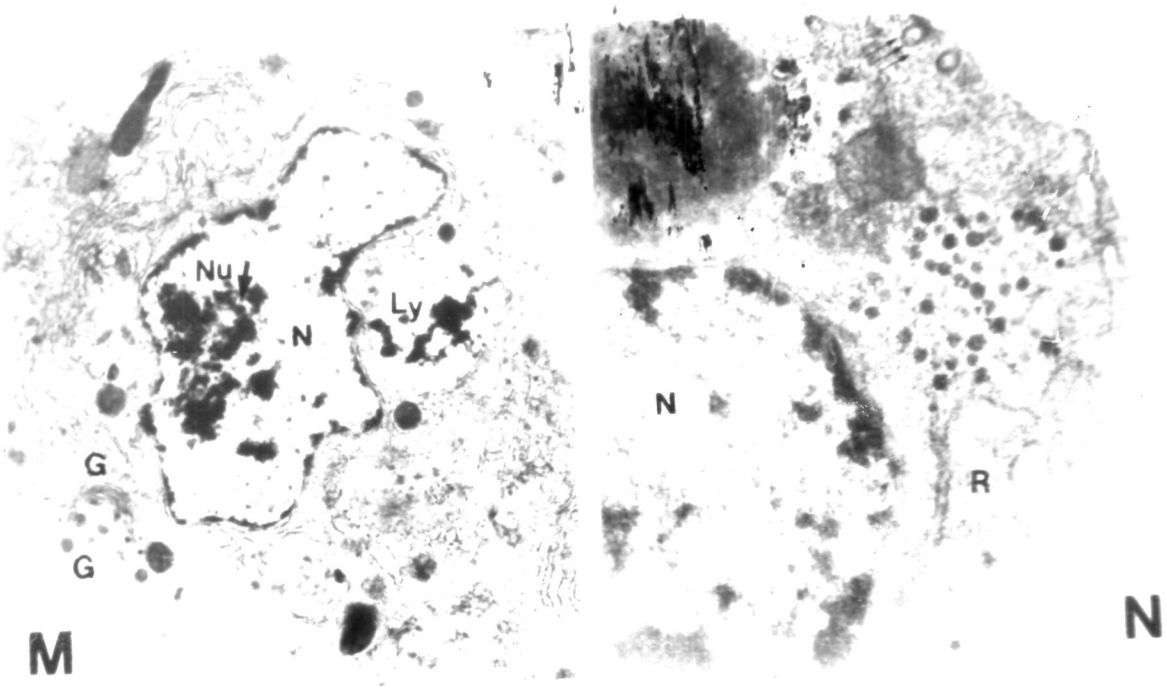
Fig. A. Granulocyte with lysosome, stacks of endoplasmic reticulum and Golgi complex and many small developing granules, lobed nucleus and less compact nucleolus. Nucleus is surrounded by a double membrane.

Fig. B. Granulocyte showing granular deposits, pinocytotic vesicles (triple arrow) and ribosomes.

Fig. C. Showing rough endoplasmic reticulum, mitochondria, granules and vacuoles. Lysosomes showing myelinated bodies (Arrow).

Fig. D. Showing many granules, vacuoles and granular deposit.

PLATE-XIX



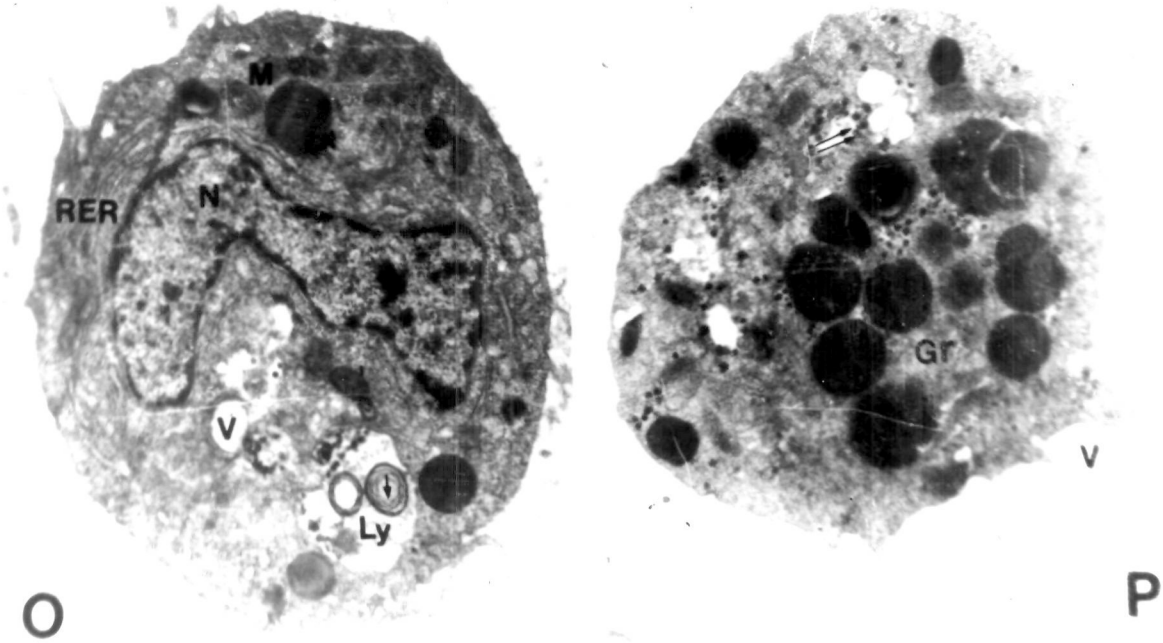


PLATE- XX

Ultrastructure of normal haemocytes under Transmission Electron Microscope (TEM), of 6th instar larvae of *Diacrisia obliqua.*

Fig. A. Oval oenocytoid showing small eccentric nucleus, fibrous homogenous cytoplasm containing a few mitochondria.

Fig. B. Round oenocytoid with small eccentric nucleus and homogenous cytoplasm.

Fig C. Showing large centrally placed nucleolus and small spheres (Double arrow) on the nuclear membrane.

Fig. D. Showing oenocytoid nucleus studded with small spheres (Arrow).

PLATE-XX

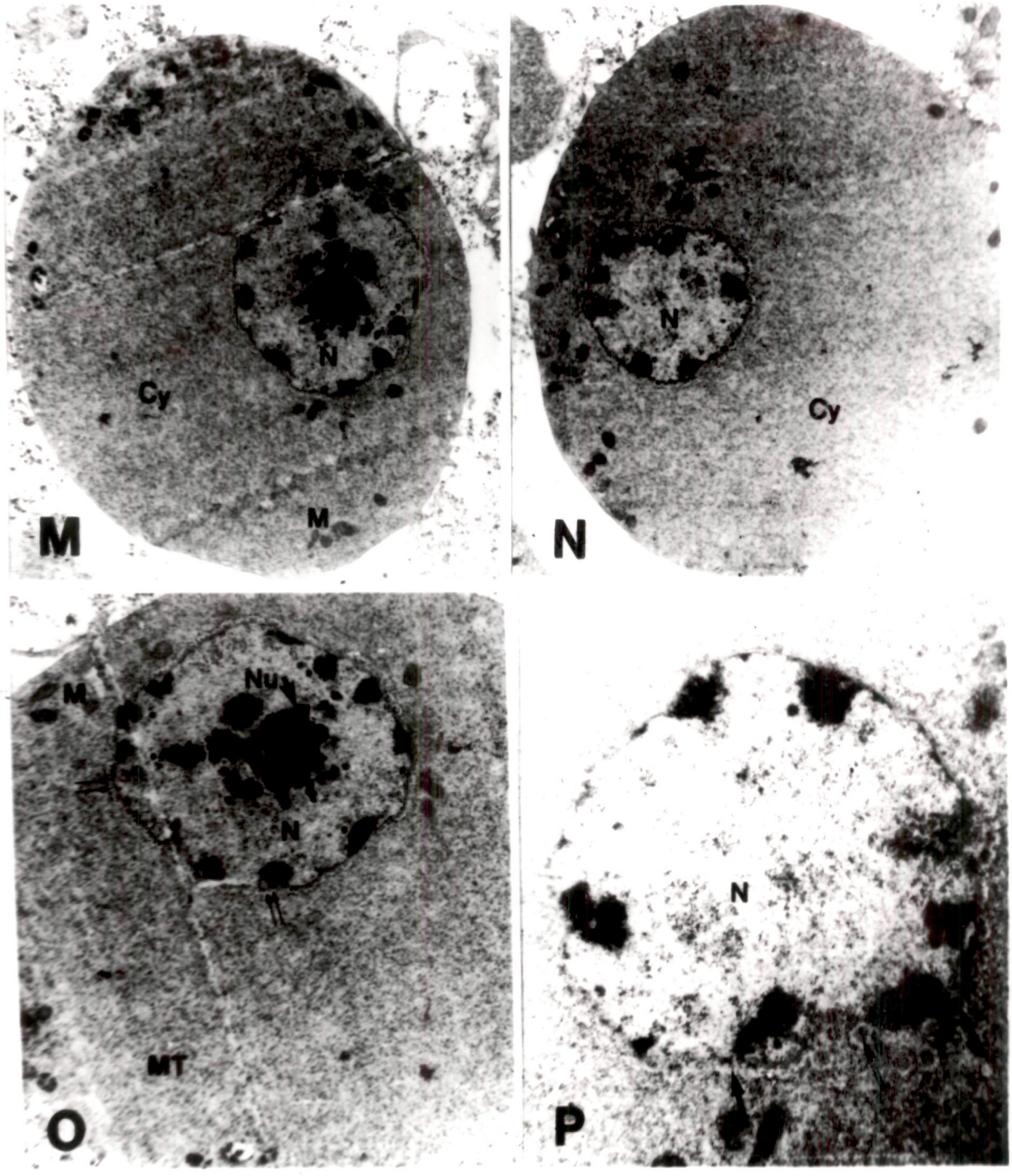


PLATE XXI

Ultrastructure of normal haemocytes under Transmission Electron Microscope (TEM), of 6^{th} instar larvae of *Diacrisia obliqua*.

Fig. A. Spherulocyte with large numerous spherule and indistinct nucleus

Fig. B. Spherulocyte with numerous spherules distinct nucleus and a phagocytic vacuoles

Fig. C. Spherulocyte with few spherules, numerous mitochondria and large network of rough endoplasmic reticulum.

Fig. D. Spherulocyte with many spherules and a well developed large nucleus

Fig. E An intermediate haemocyte showing lysosome, large mitochondria and microtubular matrix.

Fig. F. An intermediate cell showing crescent shaped nucleus, a few granules and well developed rough endoplasmic reticulum.

PLATE-XXI

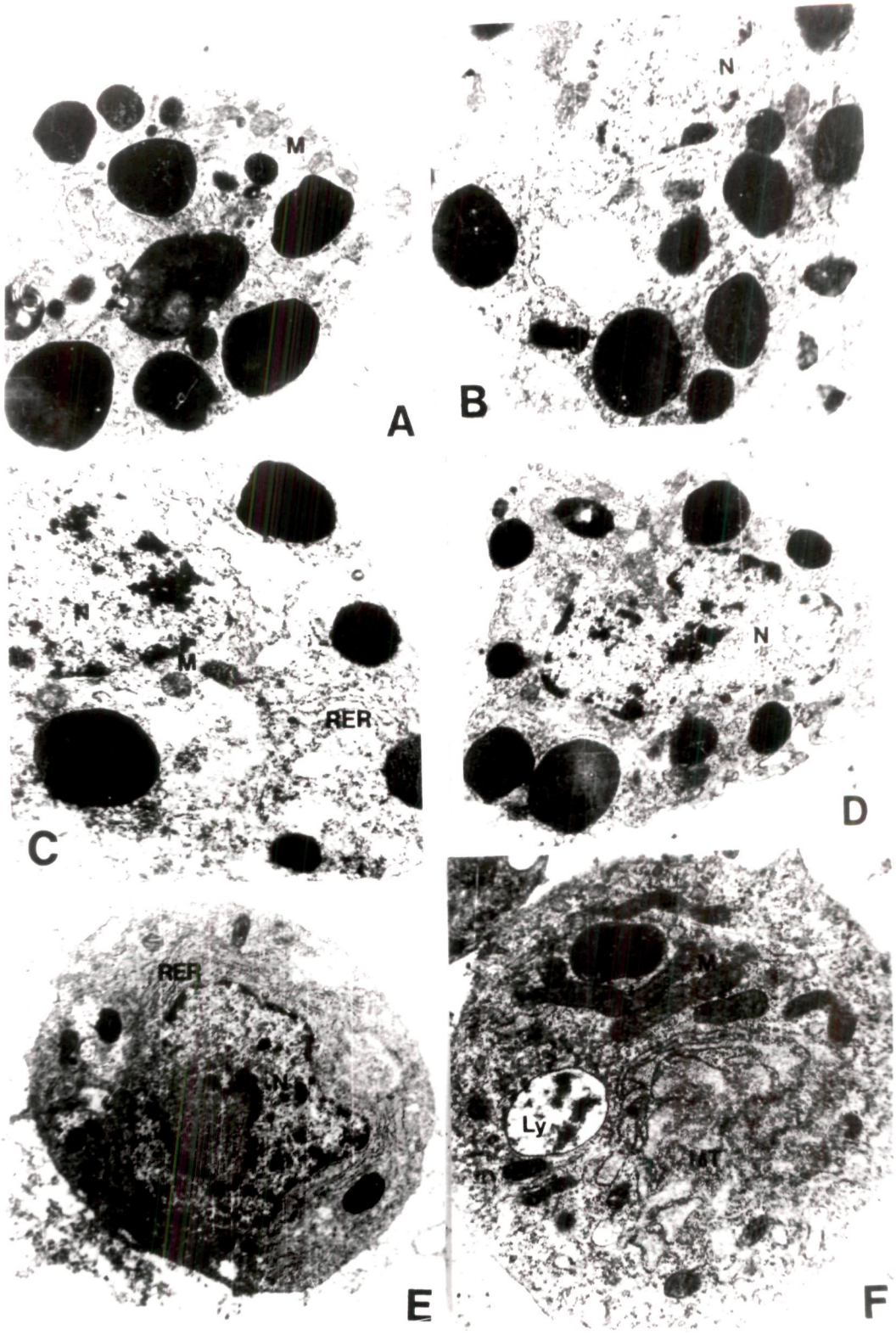


PLATE-XXII

Ultrastructure of abnormal haemocytes under Transmission Electron Microscope (TEM), of 6^{th} instar larvae of *Diacrisia obliqua* treated with various chemicals.

Fig. A. Prohaemocyte showing broken cell and nuclear membrane, and discharge of nuclear as well as cytoplasmic material

Fig. B. Magnified view of Fig. A.

Fig. C. Magnified view of Fig A showing nuclear fragments, broken cell boundary and out flow of cytoplasm.

Fig. D. Prohaemocyte showing lysed cell organelles and large nucleus with indistinct heterochromatin and euchromatin.

Fig. E. Prohaemocyte showing irregular cell surface due to formation of long tentacles.

Fig. F. Magnified view of Fig. A showing disintegration of nuclear material, subsequent formation of large vacuoles and out flow of nuclear material

PLATE-XXII

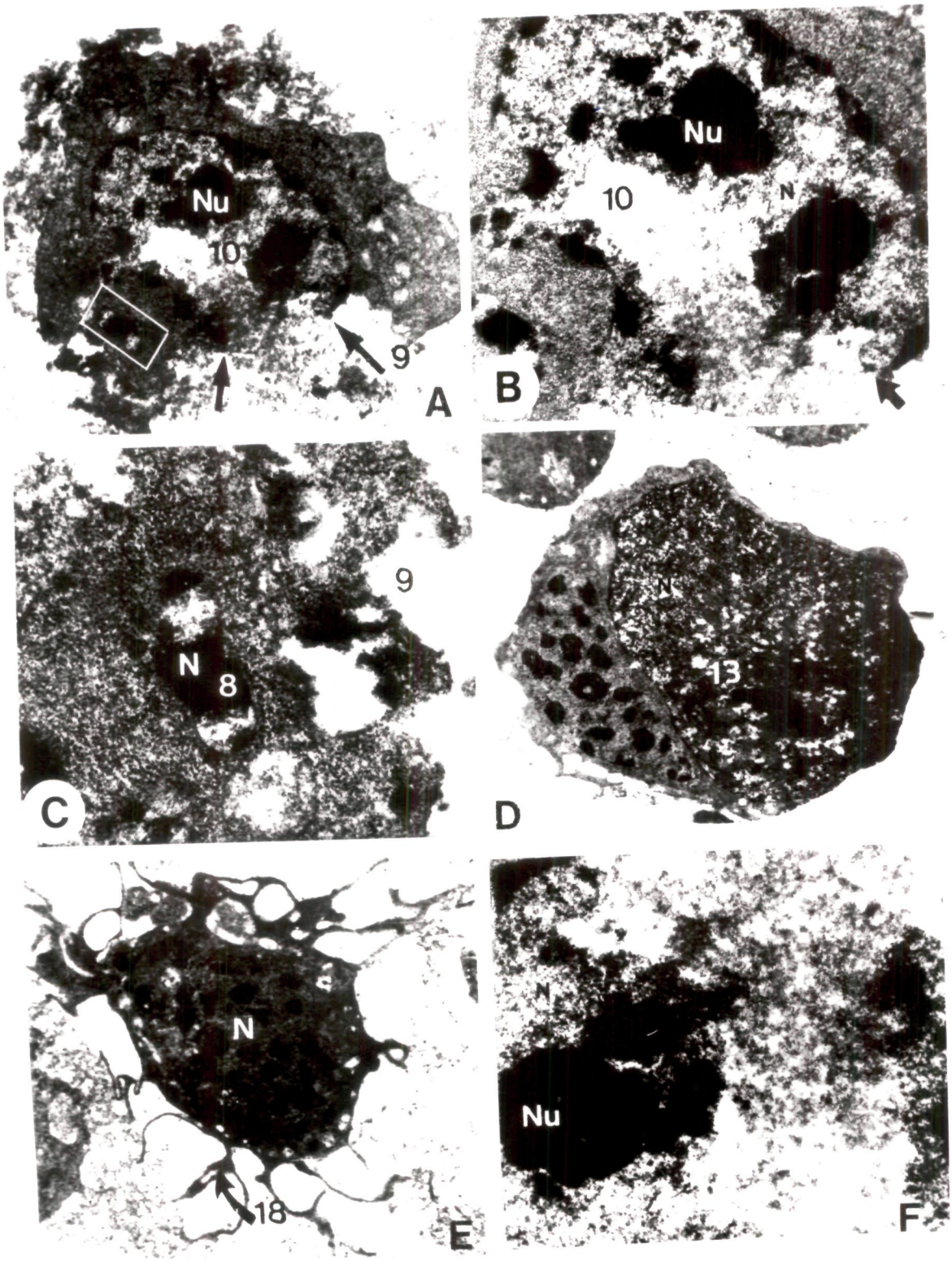


PLATE XXIII

Ultrastructure of abnormal haemocytes under Transmission Electron Microscope (TEM), of 6th instar larvae of *Diacrisia oblique* treated with various chemicals.

Fig. A & D. Prohaemocyte with irregular cell surface showing tentacular outgrowths.

Fig. B Plasmatocyte showing vacuoles and indistinct cytoplasm and nucleus

Fig. C. Plasmatocyte with irregular shape showing abnormal swelling and extension of cytoplasm.

Fig. E. Plasmatocyte with indistinct cytoplasm and nucleus containing numerous small vacuoles.

Fig. F Plasmatocyte with large swollen nucleus and stretched cell membrane.

Fig. G. Plasmatocyte showing cytoplasmic vacuoles.

Fig. H Lower magnification of Figure F

PLATE-XXIII

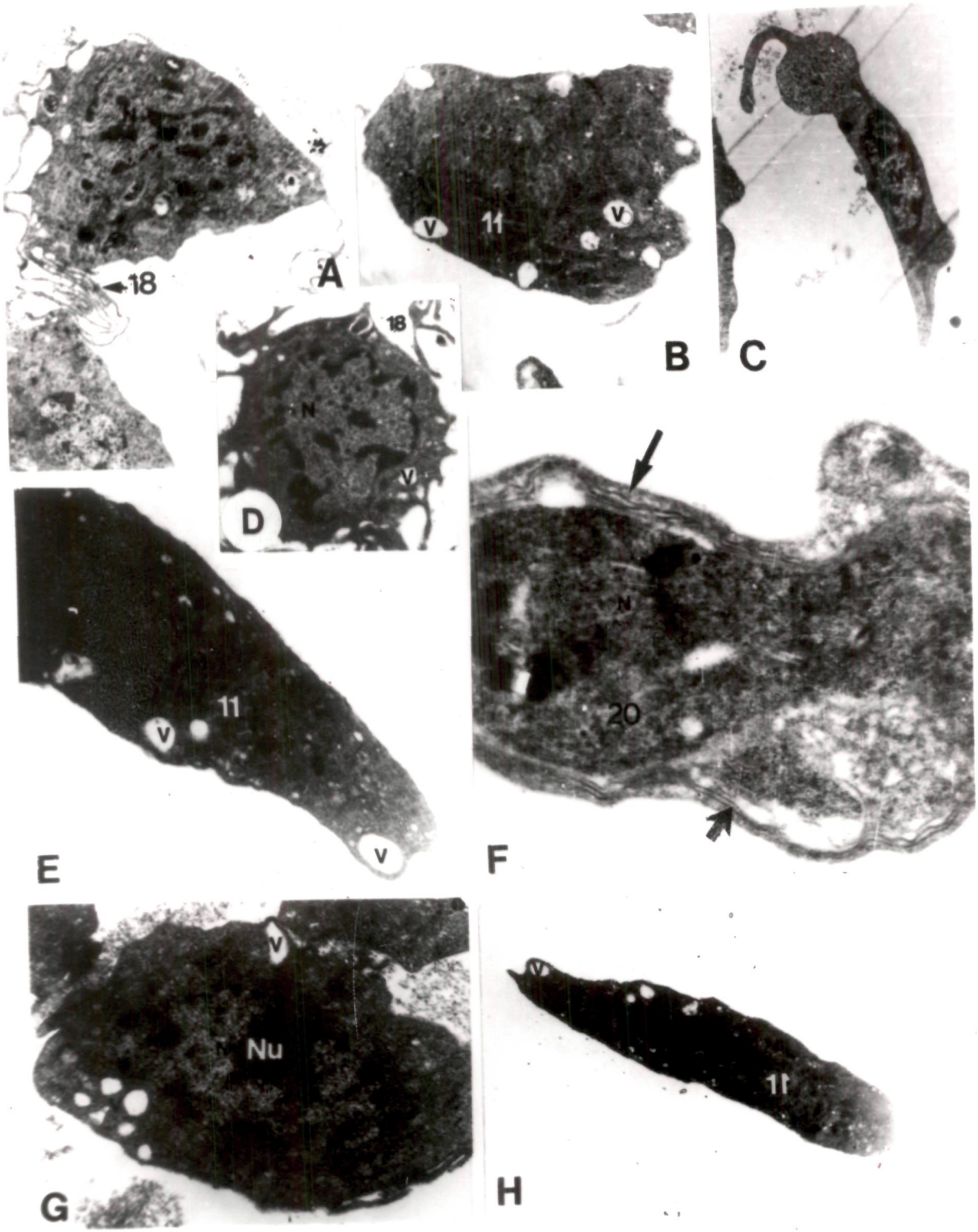


PLATE-XXIV

Ultrastructure of abnormal haemocytes under Transmission Electron Microscope (TEM). of 6th instar larvae of *Diacrisia obliqua* treated with various chemicals.

Fig A. Plasmatocyte with tentacular out growth, indistinct cytoplasm and nucleus with a few cell organelles.

Fig. B. Plasmatocyte of distorted shape showing cytoplasmic extension at one end.

Fig. C. Plasmatocyte of abnormal shape showing fragmented nucleus.

Fig. D. Plasmatocyte with irregular cell surface as well as indistinct cytoplasm and nucleus.

Fig. E. Hypertrophied mitochondria in the vicinity of nucleus.

Fig. F. Plasmatocyte with indistinct cytoplasm and nucleus containing large phagocytic vacuoles.

PLATE-XXIV

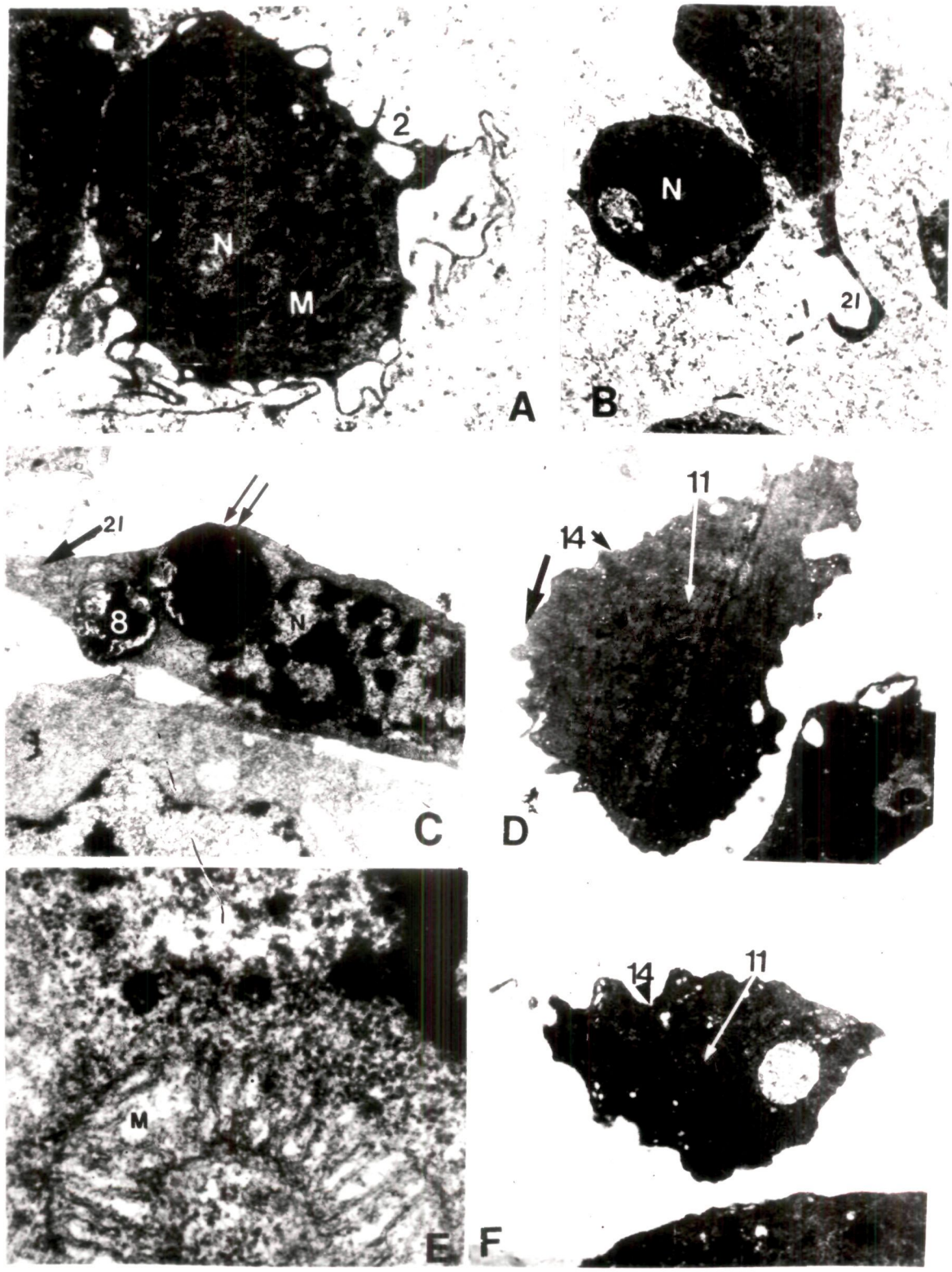


PLATE-XXV

Ultrastructure of abnormal haemocytes under Transmission Electron Microscope (TEM), of 6th instar larvae of *Diacrisia obliqua* treated with various chemicals.

Fig. A. Plasmatocyte having distorted shape and vacuoles in cytoplasm as well as disrupted nuclear membrane.

Fig. B. Plasmatocyte showing irregular cell boundary and few vacuoles.

Fig. C. Plasmatocyte with small pseudopodia like processes and indistinct cell organelles.

Fig. E. Plasmatocyte having shrinked nucleus and large cytoplasmic vacuoles.

Fig. D. Plasmatocyte with indistinct cell organelles and pseudopodia.

Fig. F. Plasmatocyte with small processes and numerous mitochondria and phagocytic vacuoles.

Fig. G. Plasmatocyte with eccentrically pushed nucleus and a electron lucent large, well rounded mass.

PLATE-XXV

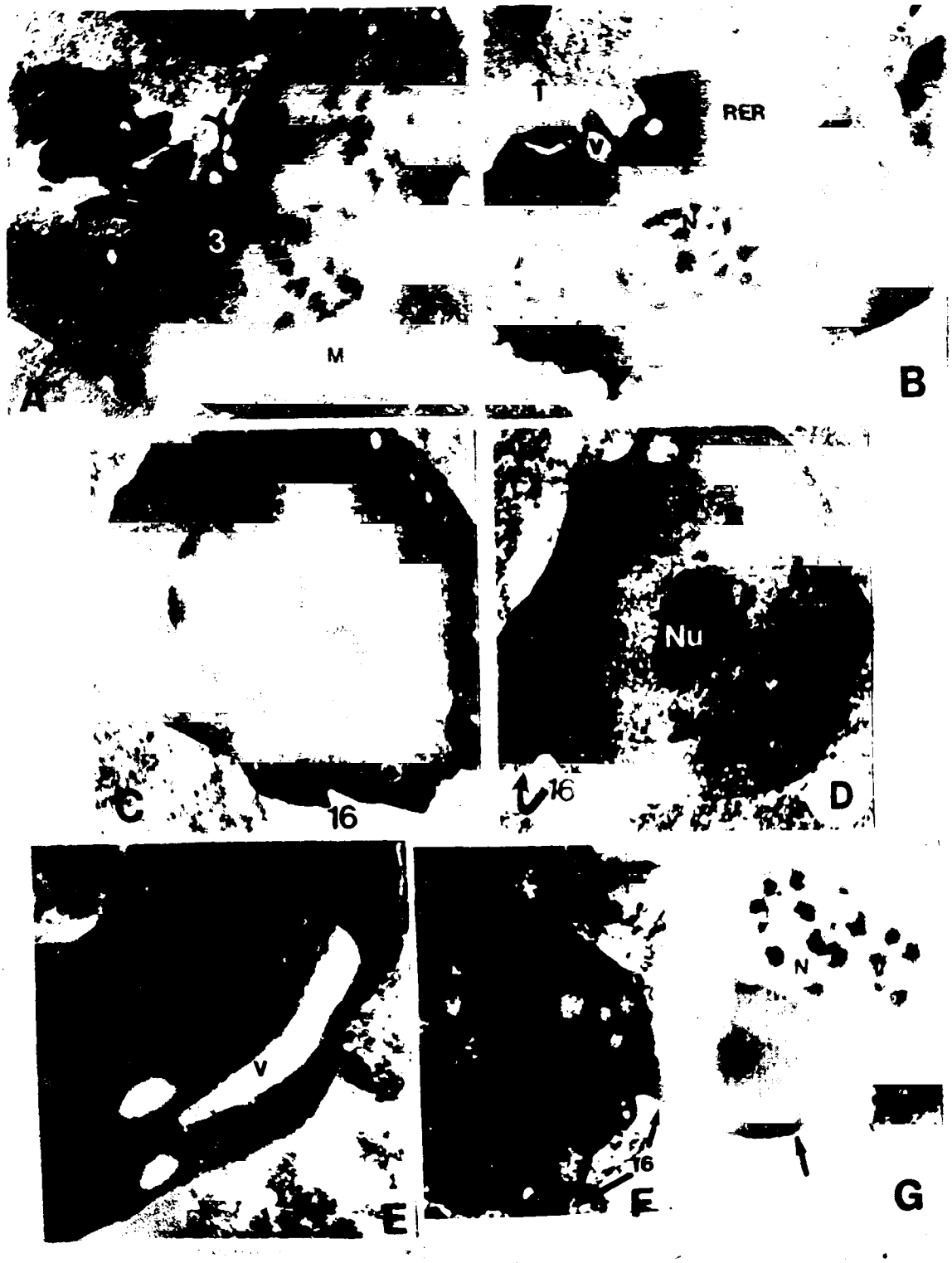


PLATE-XXVI

Ultrastructure of abnormal haemocytes under Transmission Electron Microscope (TEM), of 6th instar larvae of *Diacrisia obliqua* treated with various chemicals.

Fig. A. Plasmatocyte showing vacuoles in cytoplasm and fragmented nucleus.

Fig. B. Spindle shaped plasmatocyte with smooth cell membrane and fragmented nucleus.

Fig. C. Plasmatocyte with fragmented nucleus.

Fig. D. Disintegrating granulocyte with irregular cell membrane, few granules and autophagic vacuole the nucleus is absent.

Fig. E. Granulocytes with very small nucleus, numerous granules.

Fig. F. Granulocyte showing small nucleus and numerous granules.

Fig. G. Granulocyte showing small granules, distinct cell organelles and large nucleus.

PLATE-XXVI

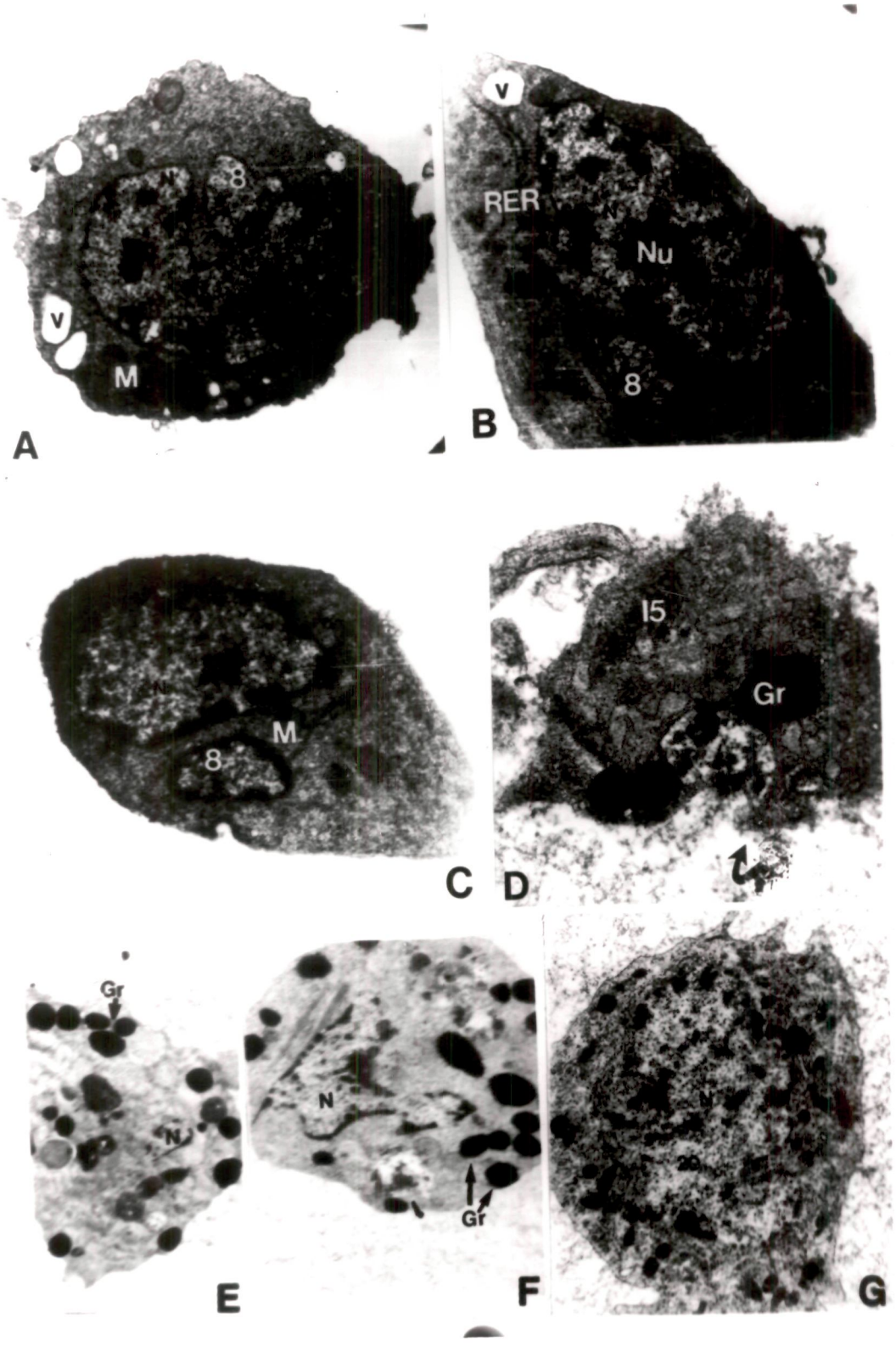


PLATE-XXVII

Ultrastructure of abnormal haemocytes under Transmission Electron Microscope (TEM). of 6th instar larvae of *Diacrisia obliqua* treated with various chemicals.

Fig. A. Granulocyte showing ragged appearance, numerous granules. Lysosome and very small nucleus.

Fig. B. Granulocyte having fragmented nucleus, phagocytic vacuole and few cytoplasmic vacuoles.

Fig. C. Granulocyte with large tentacular outgrowth on one end, large cytoplasmic vacuoles and electron dense ragged nucleus.

Fig. D. Granulocyte having large phagocytic vacuoles and very small eccentric nucleus.

Fig. E. Granulocyte having irregular cell surface, few vacuole and disrupted plasma membrane at several process. Discharge of granules to outside of cells was visible.

Fig. F. Unaffected oenocytoid showing homogenous materials, few organelles and small eccentric nucleus with smooth plasma membrane.

PLATE-XXVII

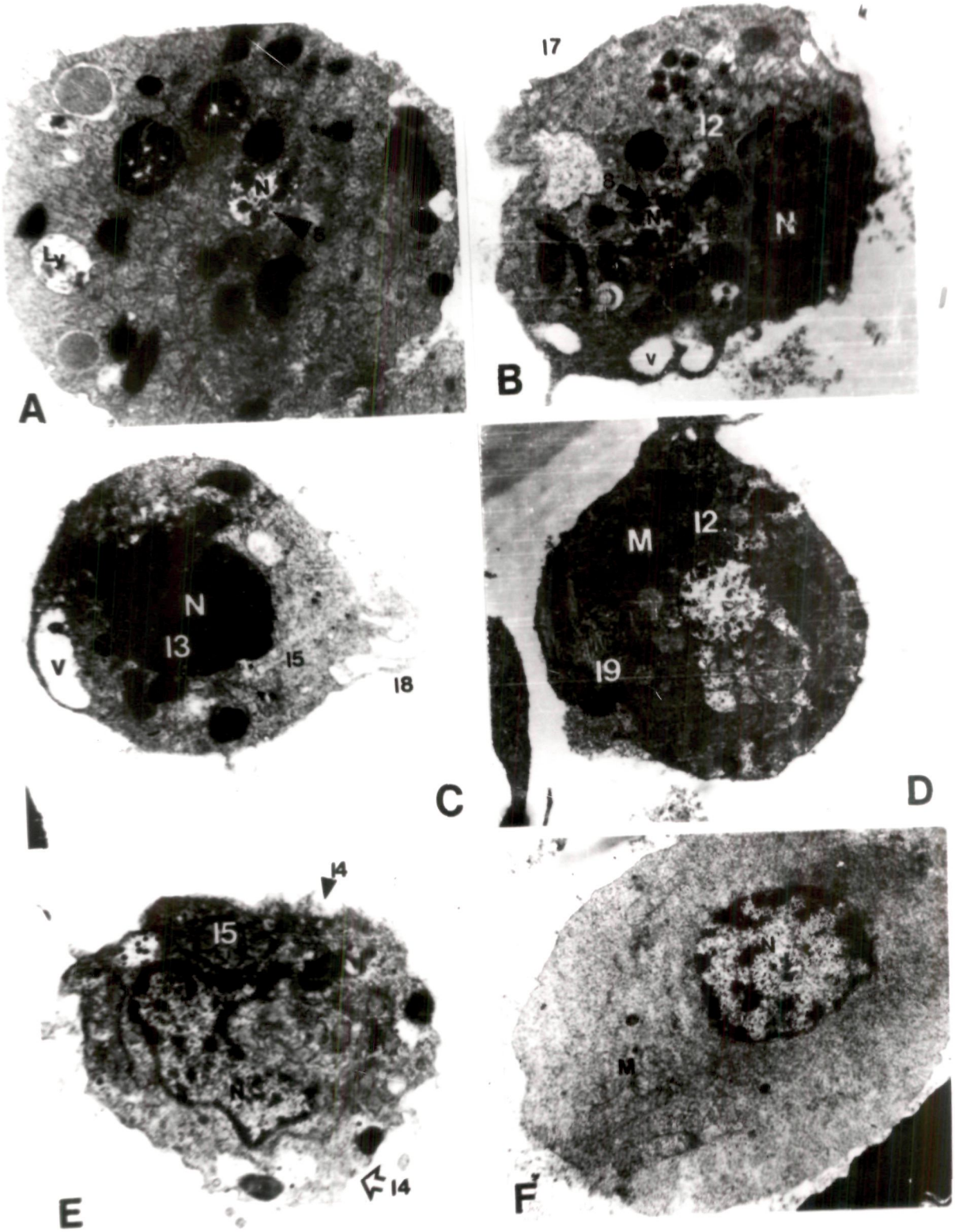


PLATE-XXVIII

Ultrastructure of abnormal haemocytes under Transmission Electron Microscope (TEM), of 6th instar larvae of *Diacrisia obliqua* treated with various chemicals.

Fig. A. Oenocytoid having large central nucleus and homogenous cytoplasm containing microtubules.

Fig. B. Oenocytoid with irregular nucleus, mitochondria and a few granules in the cytoplasm.

Fig. C. Oenocytoid showing irregular cell surface and very minute vacuoles as well as small nucleus having disrupted nuclear membrane.

Fig. D. Haemocyte showing numerous vacuoles, small eccentric nucleus and many tentacles on the surface.

Fig. E. Haemocyte showing vacuolated cytoplasm and a phagocytic vacuole as well as disintegrated nucleus.

Fig. F. Magnified view of fig. D.

PLATE-XXVIII

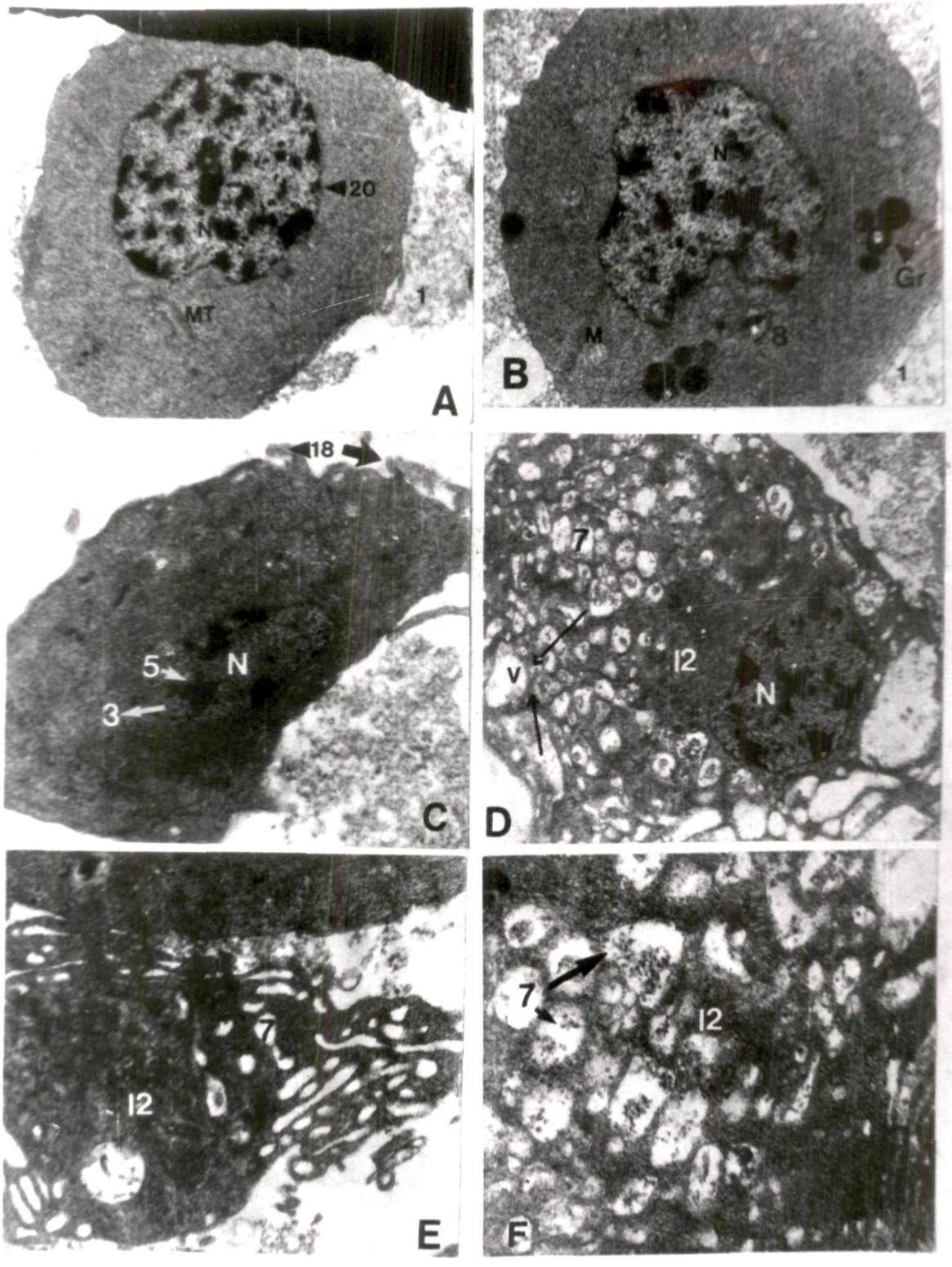


PLATE XXIX

Ultrastructure of abnormal haemocytes under Transmission Electron Microscope (TEM), of 6^{th} instar larvae of *Diacrisia obliqua* treated with various chemicals.

Fig. A.	Haemocyte with numerous small vacuoles, indistinct cell organelles and nucleus with distinct heterochromatin and euchromatin.
Fig. B.	Haemocyte with vacuoles and elongated nucleus.
Fig. C & D.	Haemocyte without nucleus showing numerous mitochondria, small granules and free ribosome.
Fig. E.	Haemocyte showing long tentacles and small fragmented nucleus.
Fig. F.	Haemocyte showing large spherule, indistinct cell organelles and numerous free ribosomes.

PLATE-XXIX

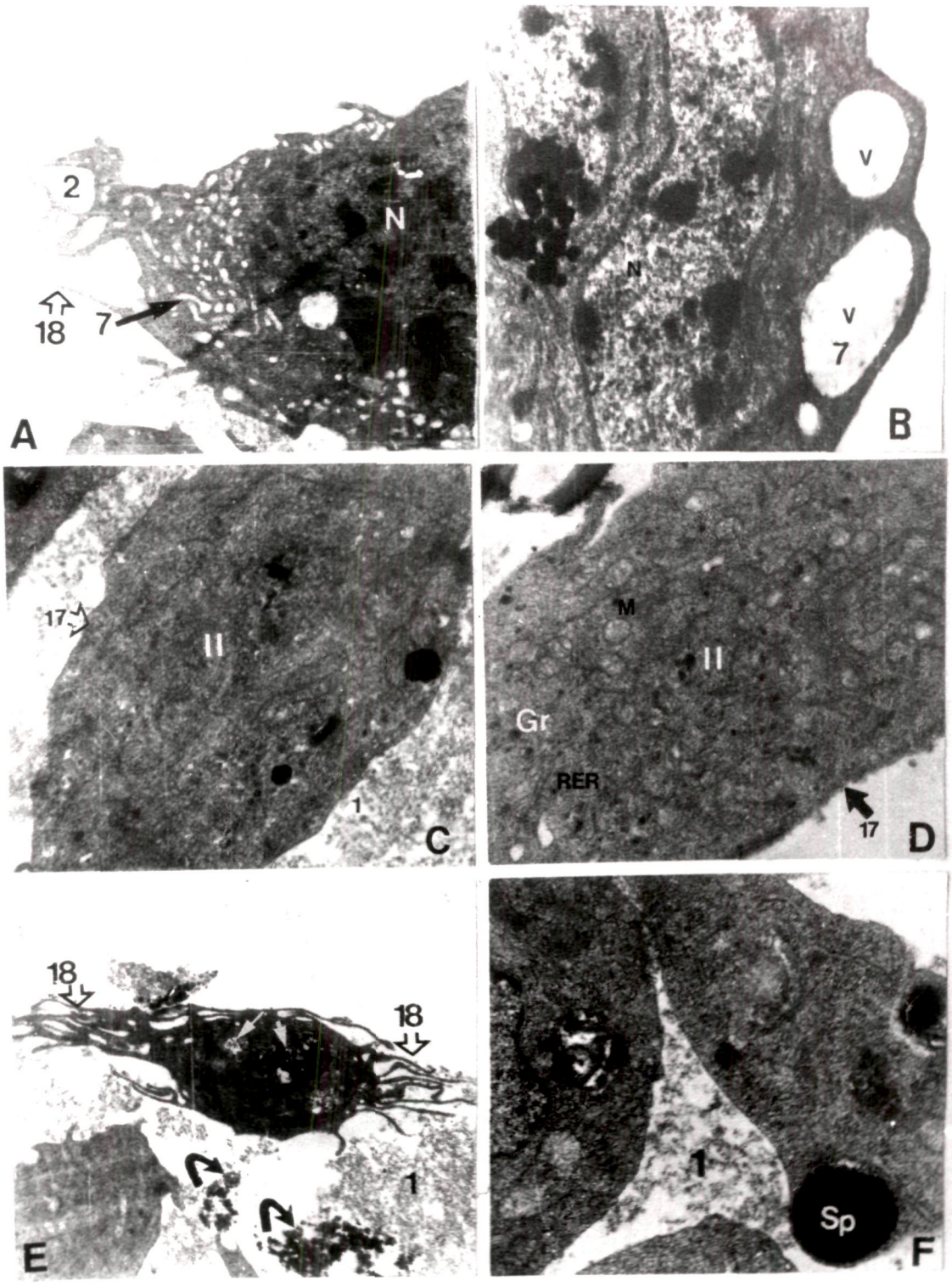


PLATE-XXX

Ultrastructure of abnormal haemocytes under Transmission Electron Microscope (TEM), of 6th instar larvae of *Diacrisia obliqua* treated with various chemicals.

Fig. A. Haemocyte showing small eccentric nucleus, spherule like inclusion and numerous free ribosomes.

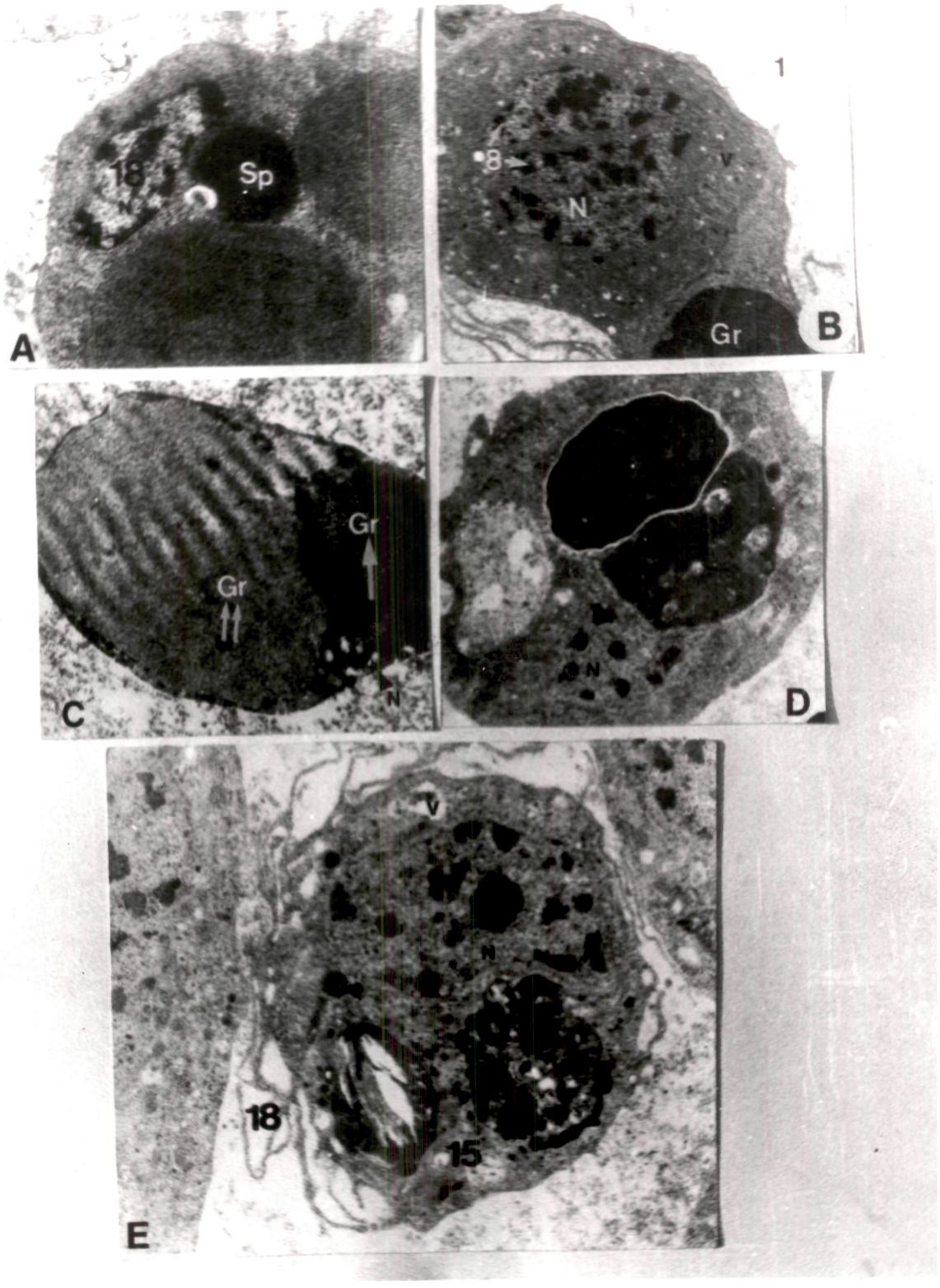
Fig. B. Haemocyte showing fragmented nucleus and extension of cytoplasm containing a large electron dense granule-like mass.

Fig. C. Haemocyte containing two large masses, one containing light coloured matrix and other electron dense, as well as small eccentric and partly eclipsed nucleus.

Fig. D. Haemocyte containing two large electron dense structures and a phagocytic vacuole as well as small eccentric nucleus with distinct heterochromatin and euchromatin.

Fig. E. Haemocyte containing large nucleus and extensive disrupted cytoplasm with disintegrated cytoplasmic organelles.

PLATE-XXX



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